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Dairy cow traits responses in dependency of breed and
environmental characteristics along a rural-urban gradient.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
AIC	Akaike information criteria
BCS	Body condition score
BIC	Bayesian information criteria
BinEpG	Binary defined Eggs per Gram
BinOpg	Binary defined Oocysts per Gram
BW	Body weight
CH ₄	Methane
DM	Dry matter
EpG	Eggs per Gram
ET	Environmental temperature
FC	Phenotypic observation,
GHG	Greenhouse gases
GIN	Gastrointestinal Nematode
GIS	Geographical information system
GLMM	Generalized linear mixed model
G×E	Genotype by environment interaction
HAS	Hock assessment score
HF	Holstein Friesian
HG	High genetic
HG	Heart girth
IRR	Index of relative rurality
IT	Information technology
KMF	Karnataka Milk Federation
LG	Low genetic
LMD	Laser methane detector
LMM	Linear mixed model
LP	Legendre polynomials
LS	Locomotion score
LSMeans	Least square means
MUN	Milk urea nitrogen
MW	Metabolic weight
MY	Milk yield

MYC	Milk yield classes
NDF	Neutral detergent fiber
NIANP	National Institute of Animal Nutrition and Physiology
OpG	Oocysts per Gram
PA	Parent average
RH	Relative humidity
RND	Pseudo-random normal deviates
RT	Rectal temperature
RUI	Rural-urban index
SCS	Somatic cell score
SD	Standard deviation
SE	Standard error
SES	Social-ecological systems
SSI	Survey stratification index
SubMast	Subclinical mastitis
T	Temperature
TBU	True breeding values
THI	Temperature-humidity index
UddHS	Udder hygiene score
ULHS	Upper leg hygiene score
URI	Urban-Rural Index
UV	Ultraviolet

SUMMARY

Rising megacities represent the highest state of urbanization, which is an important ongoing transition process in the socio-ecological field. The dairy livestock in India is based in rural and (peri-)urban smallholders, a vital livestock sector in a society that strongly relies on milk and milk products as protein sources in vegetarian diets. The social-ecological heterogeneity along rural-urban gradients in rising megacities might influence the dairy production, through phenotypic trait expressions. However, the impact of social-ecological factors along an urbanizing environment in dairy cattle had not yet been evaluated in terms of phenotypic trait expressions.

This thesis focused on the study of phenotypic trait expressions including production traits, body condition, methane emissions, cow wellbeing indicators and health traits to study the effects of urbanization on dairy production. In this regard, the rising megacity of Bangalore, in southern India, was selected for its challenging environmental conditions and social complexity. Mixed modelling approaches were applied considering both social and ecological continuous descriptors simultaneously to investigate the impact of social-ecological components on production and functional traits. A rural-urban gradient, based on an index-based approach comprising build-up density and distance to the city center, was considered as a novel environmental descriptor. Possible genotype by environment ($G \times E$) interactions are studied, considering different cattle breeds and the level of urbanization as an environmental descriptor.

Chapter 1 gave an outline of how urbanization has developed, grown and affected dairy livestock systems, especially in developing countries, with a particular emphasis on India and Bangalore. In this regard, different index-based approaches to define urbanization and social-ecological systems were explained. Furthermore, chapter 1 provides an overview of the effects of urbanization on different dairy cattle traits.

Chapter 2 developed and evaluated a method for simulating true breeding values in a multivariate framework through random regression coefficients. This approach allowed the reconstruction of missing covariances among traits or among random regression coefficients. This approach generates a single **G**-matrix, which includes all (co)variance components for all trait combinations. A brief example of a (co)variance matrix including six traits is used to describe the methodology systematically. The method described in chapter 2 can be interpreted as a backward procedure when simulating phenotypic records in a multivariate approach, and

is mainly based on overlapping elements in two sub-matrices used for generating one single **G**-matrix. (Co)variance components from the generated **G**-matrix (created on the data basis of two sub-matrices) and the original **G**-matrix were almost identical for already existing elements, and (co)variances for unknown elements had the same sign and range. Furthermore, chapter 2 extends the methodical evaluation to a multivariate random regression problem for six traits from three lactations and five random regression coefficients per trait and lactation, implying a matrix **G** of dimension 90 x 90. Two different sub-matrices **G**₁ and **G**₂ varying in dimension and in the number of overlapping elements are created, in order to reconstruct all elements in **G**. When comparing re-constructed and original elements, scenarios with one overlapping “trait square” in both sub-matrices gave the best agreement. In the last section of chapter 2, random regression coefficients for additive-genetic effects for milk-kg and body condition score from different studies are combined with fixed trait curves in dependency of the social-ecological gradient in Bangalore. Generated phenotypic curves for milk-kg and body condition score in first lactation represent the expected shape from the raw phenotypic data. In addition, simulated daily cow records can be multiplied with daily costs and revenues, in order to determine dairy cow efficiency or to derive economic weights for specific social-ecological levels.

Chapter 3 addressed associations between a continuous rural–urban gradient and phenotypic trait expressions. The phenotypic dairy cattle traits included in the study were a combination of production traits (daily milk yield: MY), body fat reserves indicators (body condition score: BCS), cow wellbeing indicators (udder hygiene score: UddHS, upper leg hygiene score: ULHS, hock assessment score: HAS, rectal temperature: RT), and health traits (locomotion score: LS, subclinical mastitis: SubMast). MY and BCS were higher in urban than in rural areas, associated with reduced SubMast and improved hygiene scores for UddHS and ULHS. Scores for wellbeing indicators HAS and LS were unfavorable for cows in urban areas, indicating poor leg health conditions in that area. In rural areas, least-squares means for RT were quite large, probably due to the scarcity of shading and heat insulation of the sheds. Thus, findings in chapter 3 corroborated differences in phenotypic trait expressions in dairy cows along the rural-urban gradients of Bangalore. The use of a rural-urban index in trait analyses contributes to a deeper understanding of cow trait reactions on social-ecological challenges underlying G×E interaction.

Chapter 4 focused on the analysis of CH₄ concentration in air exhaled by dairy cows along the rural-urban gradient of Bangalore using a laser methane detector (LMD). Mean, maximum and CH₄ concentration per duration of the overall measurement, eructation and respiration bouts

were calculated individually at cow level. For the overall mean and respiration bouts, CH₄ concentration was higher in cows from urban areas, which had also higher MY than cows from (peri-)urban and rural areas. Consideration of different dairy husbandry systems (feeding strategy, farm location and shed type) contributed to a deeper understanding of enteric CH₄ emission variations in an urbanizing environment. The LMD measurements corroborated differences in enteric CH₄ emissions in dairy cows along the rural-urban gradient of Bangalore, indicating the associations between animal greenhouse gas emissions and social-ecological challenges.

Chapter 5 investigated social-ecological effects on gastrointestinal nematode (GIN) and *Eimeria* spp. infections in dairy cattle along the rural-urban gradient of Bangalore. The level of urbanization at the farm location significantly influenced the probability of *Eimeria* spp. infection with higher infection probability in rural areas. The physiological status of the cattle also influenced the infection probabilities, both GIN and *Eimeria* spp. infections were higher in calves and heifers compared to lactating and dry cows. The variations in endoparasite infection intensity and probability along the rural-urban gradient reflected the variability in dairy husbandry systems governed by the social-ecological context. Moreover, chapter 5 suggests that the close cohabitation of humans and livestock as a result of rapid urbanization encourages better animal health management, but simultaneously increases the risk for specific parasitic infections depending on the parasite's life-cycle.

Finally, **Chapter 6** gave a general discussion focusing on the most pertinent results from chapter 2 to 5. Furthermore, some concerns on social-ecological challenges on herd management and environmental conditions that influence the productivity and functionality of dairy cattle were discussed. Additionally, G×E interaction was detected along a novel environmental descriptor, an urbanization index-based approach, for both production and functional traits. These findings are important for creating more complete and accurate socio-ecological models for the analysis of livestock production systems in urbanizing environments.

CHAPTER 1

General Introduction

1. Urbanization

The future of the world's population is urban (United Nations, Population Division, 2019a). In 2007, the world reached a momentous milestone: for the first time ever, the world's urban population exceeded the rural population – a fact which is expected to continue to grow, up to a two-thirds ratio by 2050 (Ritchie, 2018). This urban population growth is caused by both natural causes (more births than deaths) and by migration (from rural to urban centers), causing cities to expand their boundaries and the reclassification of former rural settlements as urban districts (Satterthwaite et al., 2010).

The most advanced form of urbanization is represented by megacities, metropolitan areas with a total population of more than 10 million inhabitants. Megacities have been described as the urban phenomenon of the 21st century (European Strategy and Policy Analysis System, 2018). Most of these urban agglomerations are in developing regions. India is a prominent example for megacity development, already having six megacities (Bangalore, Chennai, Delhi, Hyderabad, Kolkata and Mumbai); this means India has the highest concentration of megacities in the world (Brinkhoff, 2020).

1.1. Urban and (peri-)urban agriculture: A path to sustainable urbanization

As a result of urbanization, cities are growing; so too grow not only the challenges (land scarcity for agriculture purpose, carbon emission associated to climate change and risk of zoonosis) but also the opportunities to make cities more sustainable. The Food and Agriculture Organization of the United Nations (FAO, 2019), in its framework for the urban food agenda, recognizes the need to consider food security and nutrition as key elements in planning for sustainable cities and proposes the promotion of urban agriculture as a way to achieve this. Urban and (peri-)urban agriculture contribute to improvements in food supply, food security, local economies, social integration and environmental sustainability in urbanizing environments. However, urban and (peri-)urban agriculture will not be able to meet 100% of a city's food requirements, rather they serve to complement the supply from rural agriculture, primarily by providing perishable products such as vegetables, milk and eggs (Orsini et al., 2013).

1.2. Livestock production in urbanizing environments

Urbanization leads to rapid income growth in urban areas which, in turn, tends to change the diet and to increase the demand for high protein foods – mainly of animal origin (meat and dairy products) (Delgado et al., 1999). This increasing demand for animal-source food will

mean that livestock production will likely play a key role in achieving sustainable food security in developing countries, in the context of rapid urbanization (Godber and Wall, 2014). Unfortunately, livestock production in urbanizing environments faces its own challenges such as the need to increase production to meet rising demand while concurrently reducing carbon emissions. In addition, livestock production has to compete for increasingly scarce natural resources as land and water are allocated to the production of human food and biofuels (Thornton, 2010). The increase in intensive livestock production near urban areas has both advantages (reduced transport distance and therefore CO₂ emissions, increased opportunities for inclusive local supply chains and improved access to fresh food through farmers' markets) and disadvantages (increased pollution of land and water can have negative effects on human and animal health) (McDermott et al., 2010). Although livestock production has so far managed to meet the demands of a growing population with less labor force and it is moving towards more intensive food production (Satterthwaite et al., 2010), there are increasing uncertainties about how livestock production systems in developing countries might evolve under urbanization dynamics and their ability to meet the growing demand for animal-source food in a sustainable manner (Abu Hatab et al., 2019; Herrero et al., 2016).

1.2.1. Effects of urbanization on different dairy cattle traits

In developing countries, meat consumption strongly depends on sociodemographic variables, such as age, education, gender and especially religion, that prohibits the consumption of certain types of meat (e.g. pork in Islam or beef in Hinduism) or promotes a certain type of diet (vegetarianism in Jainism). However, the consumption of milk and dairy products is welcome in most religions and social strata, with dairy cattle farming playing one of the most important roles in the nutrition of developing countries.

As developing countries are more seriously impacted by volatilities in food production and distribution, it is crucial to maximize their output in the most efficient way possible. There are many factors that impact this efficiency. Productivity, fertility and health of dairy cattle are directly related to farm management. However, management strategies for dairy farming can vary depending on factors such as feed availability, social categories, land availability and household income which vary depending on the location of the farm (urban, (peri-)urban or rural).

To study dairy management efficiency in different locations, Manivannan and Tripathi (2007) surveyed 150 dairy households from urban, (peri-)urban and rural localities of southern India.

He found that urban dairy farmers in southern India are better at planning, decision making, risk taking, coordination and rational marketing. They also tend to adopt more dairy husbandry practices compared with rural and (peri-)urban areas. In his study, Mannivannan and Tripathi also found that the strongest effect was between the economic motivation of dairy farmers and management efficiency, meaning that the farmers who farmed for money were more likely to engage in efficient management practices; this was followed by knowledge level about improving dairy husbandry practices in urban areas. Moreover, the variable herd size also showed a positive relationship with management efficiency in urban areas indicating that as the number of dairy animals per herd increases, the managerial efficiency of the dairy farmers also increases, with the aim of maximizing their returns.

This focus on a larger herd size in urban areas compared to rural areas has been found in various other studies as well. In a study in Assam, India, Deka et al. (2020) found that the majority of large farms (> 10 dairy cattle) were located in urban areas (78%) while farms of a medium (4-10 dairy cattle) and small (1-3 dairy cattle) herd size were more common in (peri-)urban and rural farms. Similarly, in Gondar, Ethiopia, Demlie et al. (2020) found a mean difference of almost 3 dairy cattle between herds on urban and (peri-)urban dairy farms (16.94 vs 14.15 respectively). This difference was also reflected in Northern Ethiopia where, despite a smaller overall herd size, herds were larger in urban (6.78 dairy cattle) compared with (peri-)urban areas (4.83 dairy cattle; Weldeslasse and Gangwar, 2015).

Although some studies, such as those by Abera (2016) and Bainesagn (2016), have found a larger herd size in rural areas (11.67 cattle) compared with urban areas (9.88 cattle), a closer analysis of the data shows that rural numbers were inflated due to having higher numbers of draught animals and non-productive animals, such as bulls, oxen, heifers or calves; this suggested a reduced focus on production-orientated farming in rural areas. Thus, after accounting for these adjustments, the percentage of lactating cows was higher in urban (28-31%) followed by (peri-)urban (27-28%) and then rural areas (20-25%); this supports the concept that the herd size is larger in urban areas resulting in increased efficiency of dairy production and management, as suggested by Manivannan and Tripathi (2007).

Conversely, Gizaw (2017) considered genetic variation in farms in East Africa, finding that the number of dairy cattle with 75% or above exotic genes were higher when approaching the cities. He therefore concluded that the inclusion of higher yielding breeds (e.g. Holstein Friesian or Jersey) was used as a formula for creating production-oriented dairy farming in urban areas. Similarly, Woldegebriel et al. (2017) observed better management and productivity in urban

farms through herd composition (more productive animals and higher yielding breeds). In the capital of Lesotho, urban farms showed higher average milk yield per cow per year (1829 kg) compared with rural areas (730 kg; Woldegebriel et al., 2017). This suggests that herd composition and therefore herd management are related to farm location in urbanizing environments.

Although urban farms show higher milk outputs per animal, they use more inputs too such as feed or veterinary care (Patil et al., 2019; Woldegebriel et al., 2017). The better availability of these inputs facilitates efficient planning, easy mobilization of resources, timely adoption of innovations and better management of the dairy farm (Manivannanan and Tripathi, 2007). Increased access to veterinary care results in improved fertility in urban areas by improving cattle heat detection, decreasing the number of artificial insemination (AI) services, shortening the age at first insemination and the calving interval (Gizaw et al., 2016). This suggests that the level of urbanization affects the accessibility of inputs such as veterinary care, which could have an impact on dairy cattle traits.

Land availability is another factor that impacts management strategy in dairy farming, especially as limited land can negatively impact on dairy cattle traits. A dairy farming system study in urban and (peri-)urban areas of Cairo city, Egypt (Daburon et al., 2017), showed two type of systems there – an integrated crop-livestock system and an indoor livestock system. Due to space constrains, the indoor livestock system is particularly common in urban areas. A herd confined indoors can present numerous health risks, like subclinical mastitis (Abrahmsén et al., 2013), hoof health (Black et al., 2017) or heat stress (Smid et al., 2018). Overcrowded stables are an ideal environment for spreading disease if a high level of hygiene is not maintained. However, increased barn cleaning frequency (Bainesagn, 2016; Vaarst et al., 2007) and better hygienic practices at milking (e.g. washing the udders and vessels before milking; Gizaw et al., 2016) were already found in urban and (peri-)urban areas compared to rural areas. The tight cohabitation of humans and animals, and the poor sanitary farming conditions in cities, leads to zoonosis development such us bovine tuberculosis or brucellosis (Daburon et al., 2017) and to the increase of gastrointestinal parasites due to the intensification of grazing in small areas (Mhoma et al., 2011; Mondal et al., 2000).

It has been argued throughout this section that the level of urbanization where the farm is located (it could also be called "environment") affects the management practices. Further, it has been shown that these management practices influence dairy cattle traits such as productivity,

fertility and health. Therefore, the level of urbanization is a key factor which should be considered when studying dairy cattle traits in urbanizing areas.

1.2.1.1. Genotype by environment (G×E) interactions

As mentioned above, the use of AI has brought great economic benefits to cattle farming around the world – by improving the productivity of dairy cattle; AI can allow a single bull to beget offspring in numerous countries, so the best genetics and genotypes are spread wider and faster throughout the world. However, despite the similarity in genotype, the offspring could exhibit different performance based on differences in climatic or management conditions; this has been identified in the literature as the interaction of genotype by environment (G×E).

A G×E interaction exists, therefore, if the same genotype develops different phenotypes in different environments. However, it should be noted that G×E interaction is of reduced importance if the differences between genotypes vary between environments without this variation causing a change in their ranking (Hammami et al., 2009) – this is called the scaling or non-crossover effect (Figure 1.1(a)). Rather, G×E interaction is more important when two genotypes rank differently in different environments, the so-called crossover or re-ranking effect (Figure 1.1(b)).

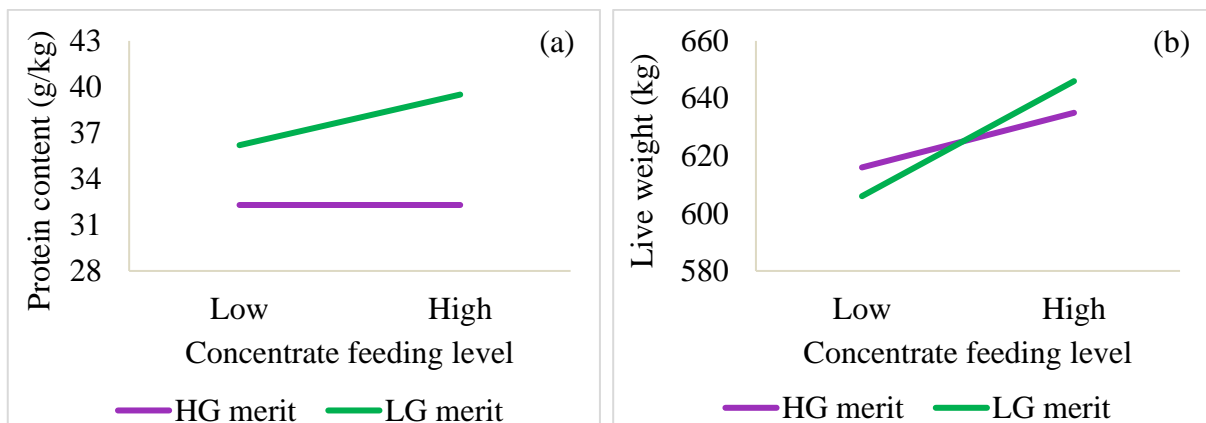


Figure 1.1. Genotype × environment interactions. (a) Non-crossover or scaling effect for protein content at first AI (g/kg) in dairy cattle. (b) Crossover or re-ranking effect for live weight at calving (kg) in dairy cattle with high genetic merit (HG merit) and low genetic merit (LG merit) in systems with low or high concentrate feeding level (Kennedy et al., 2003).

Robertson (1959) recommended a genetic correlation threshold of 0.80 as an indicator for G×E interaction, thus, genetic correlations above 0.80 show minor or no evidence for a G×E interaction. Most of the genetic correlations for production traits under different environments are above 0.80, indicating either only minor G×E interaction or a completely absence of it. However, it is more common to find evidence of G×E interaction in functional traits (Montaldo et al., 2017).

The influence of G×E is a relatively recent variable of interest in research. The most heavily researched environmental variables are region, country, management and feeding system (Carabaño et al., 1990; Kearney et al., 2004; König et al., 2005; Ojango and Pollott, 2002; Rekaya et al., 2001; Tsuruta et al., 2015). As an innovative research focus, ‘level of urbanization’ (urban vs rural) is starting to be examined in studies of human health. Kim et al. (2014) found a G×E interaction when measuring obesity traits (e.g. suprailiac skinfold thickness) between urban and rural settings in Korean individuals. The exposure to different environments (urban vs rural) affected the outcome of obesity traits, with higher obesity in urban areas. Moreover, the level of urbanization has also been used recently as an environmental variable for the study of evolutionary biology. Theodorou et al. (2018), detected patterns of genetic differentiation in the red-tailed bumblebee (*Bombus lapidaries* L.) strongly associated with urbanization. Theodorou et al. (2018), identified a G×E interaction for genes associated with metabolism, heat stress and oxidative stress, all in relation to urbanization, suggesting an adaptation to urbanization by the red-tailed bumblebee. Thus, it is clear that a G×E interaction is present in many biological systems and thus it should be considered in research on dairy farming as well. Thus, we must consider SES in the context of our increasingly urbanized world.

2. Social-ecological systems

More than two decades have passed since the concept of social-ecological systems (SES) became a framework for the intertwined study of society, in terms of the interaction between the social–economic system and the natural system, emphasizing the concept of ‘humans as a part of nature’ (Berkes and Folke, 1998). Since then the SES concept has become an emerging approach in scientific research (Cumming et al., 2015; Lescouret et al., 2015).

2.1. Social-ecological systems in urbanization

Several studies have focused on the SES approach in order to unravel the complexity of the topics involved, such as urbanization. Wu (2014) proposed one such social-ecological framework to discuss spatial and temporal patterns of urbanization. In Figure 1.2, Wu (2014) considered the relationships between biodiversity, ecosystem processes, ecosystem services and human well-being in an urban landscape and how their relationships change in time and space as a way of understanding and improving the sustainability of an urban landscape.

Due to their complex and dynamic social-ecological nature, rising megacities are considered to be a testing ground for monitoring human–environment interactions and urbanization, and as a

global experiment on sustainability (Kraas, 2007; Wu, 2014). Ostrom (2009) proposed cross-disciplinary research as a means to accumulate and increase the knowledge needed to enhance their sustainability.

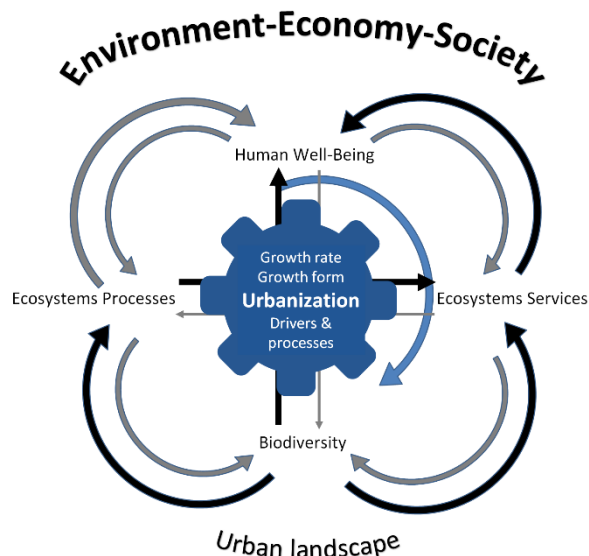


Figure 1.2. A conceptual diagram illustrating the relationships among biodiversity, ecosystem processes, ecosystem services and human well-being in an urban landscape. Adapted from Wu (2014).

2.2. Social-ecological systems in agriculture with special emphasis on livestock

Agriculture and livestock systems are the oldest and most complex examples of SES, in which societal and environmental components (humans and nature respectively) mutually depend on each other in a dynamic process shaped by uncertainty, errors, learning and adaptation. There are two research groups which have used SES models specifically in their analyses of livestock production – McDermott et al. (2010) and Duru and Therond (2015). In the McDermott et al. paper (2010) the social dimension had three components – income, livelihood and equity issues, for five groups – producers, service providers, employees, market agents and consumers; the environmental dimension considered six components – the efficient use of land, efficient use of water, efficient use of limited nutrients, minimizing the production of greenhouse gases, minimizing environmental pollutants and finally minimizing disease risks. Duru and Therond (2015) defined the livestock system as a local SES embedded in a complex multi-level and multi-domain system. Of specific importance was the idea that livestock sustainability must be based on 3 pillars: environmental, economic and social, which should be used to maintain levels of livestock production that preserve the capacity of the ecosystem. From both of these examples, it is clear that both social and environmental components, and maybe even economic components, must be analyzed in tandem rather than in isolation.

When practically applying these SES interactions, it is the ability to transform and adapt to change which is one of the core properties of SES (Ingrand et al., 2017); this adaptability is termed resilience. Livestock production is undergoing rapid changes as a result of growing global demand for high-value food products and continuous adjustments in resource use intensity, climatic risks, product orientation and marketing channels (Steinfeld et al., 2006). The challenge, therefore, for livestock producers is to ensure sustainability for their production system in this changing environment. Duru and Therond (2015) used well-identified sustainability indicators for livestock systems to assess sustainability performances. His findings identified a few key features in four dimensions which relate to the resilience of livestock systems: those were social (consumer behavior and social expectations about agriculture), political (regulations and norms), economic (level and variability of input and output prices), and ecological (climate change and animal health) systems. These changes can correspond to fast and drastic changes (e.g. price volatility, strong drought, and economic crises), or to continuous but less intensive changes (e.g. climate change). Thus, the combined use of SES with resilience is necessary when designing for sustainable agriculture (Rivera-Ferre et al., 2013).

3. Index-based approaches to define urbanization and social-ecological system

Since urbanization has begun to be recognized as a major global challenge, many studies have tried to define and characterize the rural-urban interface using discrete categories, such as urban, mixed urban, mixed rural and rural. Some have relied on economic, social, and political indicators (e.g. Isserman, 2005), others on land use allocation from government planning offices (e.g. Ghelfi and Parker, 1997). More advanced analyses have employed index-based approaches, which are quantitative and continuous measures of land use (rather than discrete categorization), to avoid the problems that arise from using arbitrary thresholds to enforce discrete category boundaries.

Some of these continuous indexes examine demographic data. Waldorf (2006) developed one such index – the “Index of relative rurality (IRR)”. The IRR is based on four factors: population size, density, percentage of urban residents and distance to the closest metropolitan area. The index varies from 0 (totally urban) to 1 (totally rural). Lin et al (2012), in another such example, suggested the creation of a coordinated index of urbanization and environment, arguing that urbanization reduces the carrying capacity of the environment and leads directly to tremendous pressure on the urban environment. They also showed that social aspects have the highest influence on urbanization compared to economic, spatial and demographic aspects and so they

suggested adding social aspects (e.g. the number of public transportation vehicles per 10,000 people and the number of doctors per 10,000 people) to indices when describing urbanization. However, when demographic data is scarce or not available, geographical information through remote sensing data is a suitable alternative.

Some indexes also attempt to incorporate geographical data in the analysis. Schlesinger (2013) developed a “Urban-Rural Index (URI)” based on two sub-Saharan African cities. The URI was calculated based on building density and travel times from the city center as the basis for analysis. The URI varies from 0 to 1 in the same way that Waldorf (2006) defined, however it too has some limitations: its calculation requires high resolution satellite images and advanced skills in geographical information system (GIS) analysis.

Arguably the best index is from Hoffmann et al. (2017), where they used a simplification of the URI from Schlesinger (2013). The main advantage is accessibility – the “Survey Stratification Index (SSI)” (Hoffmann et al. 2017) uses publicly accessible spatial input data to stratify villages in the Bangalore metropolitan region by their degree of urbanity or rurality. The SSI was calculated using GIS analysis of satellite images and combining basic measures of building density and distance to the city center. The spatial layout patterns of the different patches and the related land use implications were also taken into account when designing the index. Thus, the SSI and the measures it is based on, bear some information on the internal social-ecological structure of the rural–urban interface of Bangalore. In line with the IRR and URI, the SSI varies from 0 (totally urban) to 1 (totally rural). The geographical approach of the SSI and the publicly accessible spatial input data used, makes SSI the most appropriate and accessible index to measure the level of urbanization for the Bangalore metropolitan area where obtaining up-to-date demographic data is a complex task.

4. India

4.1. Urbanization and consumption of dairy products

India has a population of more than 1.3 billion people (United Nations, Population Division, 2019a) and it is expected to be the world’s most populous country when India surpasses China by 2027 (United Nations, Population Division, 2019b). Not only has the overall population grown but India’s urban population has doubled in the last 60 years (World Bank, 2018) and it is expected to contribute the most to the world’s urban population, adding 416 million urban dwellers by 2050 (United Nations, Population Division, 2019a). The rapid population growth

in India is accompanied by a decline in mortality rates, an increase in income per capita and a rise in demand for animal-source foods (Rae, 1998).

Among the animal-source foods, one of the highest increases has been for the consumption of milk and dairy products, which has increased in India by about 40% in the last four decades (Kumar et al., 2014; Pingali and Khwaja, 2004). India, with one third of its population following a vegetarian diet (Government of India, 2014), is the country with the largest number of vegetarians, which means they rely strongly on milk and dairy products as a source of protein in their diet. India's dairy sector therefore plays a crucial role in meeting the demand for dairy products.

4.2. Dairy livestock production

In the past 30 years, India has tripled its milk production from 54 to 188 million tons, becoming the world's largest milk producer with 22% of the global production (FAOSTAT, 2020). However, when we omit the production of milk from species other than cattle, India lags slightly behind the USA (90 vs 99 million tons in 2018 respectively) (FAOSTAT, 2020). This is because India's milk production is also supported by the largest buffalo population in the world – 114 million heads in 2018 (FAOSTAT, 2020) – accounting for more than half of the world's domestic buffalo population, which helps India to maintain its status as the world's leading dairy producer. However, the production gap between the USA and India is narrowing due both to the increase in the number of dairy cattle and their productivity. While the Indian dairy cattle production is still only one sixth of the USA's cattle production, the Indian productivity has doubled in the last 30 years (FAOSTAT, 2020). Moreover, India has the second largest cattle population in the world (12.4%) after Brazil and the largest in dairy cattle (20.0%) which has grown by a third in the last 30 years (FAOSTAT, 2020). The vast majority of dairy cattle (80%) in India are kept in small farms of 2 to 6 cattle (International Farm Comparison Network, 2019). With more than 78 million farms, India is the country with the highest number of dairy farms (International Farm Comparison Network, 2011), an order of magnitude higher than the next place on the list (Pakistan with 7.4 million).

4.2.1. Methane emission

While the increase in dairy farming and in productivity has had the positive effect of meeting the demand for milk, it has also had the negative effect of increasing greenhouse gases (GHG) as a detrimental by-product, with enteric methane (CH₄) as the largest source emitted. Concern about the contribution of dairy farming to global warming through GHG emissions has led to a

series of studies to improve scientific knowledge on enteric CH₄ emission from dairy livestock. Such studies have shown variations in enteric CH₄ emissions due to improved management practices (i.e. amount and quality of feed intake; Hammond et al., 2009), environmental effects (i.e. season influences forage quality and pasture access; O'Brien et al., 2014) and physiological characteristics (e.g. breed, body weight and milk yield; Garnsworthy et al., 2012; Swamy and Bhattacharya, 2006). Thus, by understanding the enteric CH₄ emission, it is possible to create mitigation strategies that can have a real impact considering the large dairy cattle population of India.

4.3. Urban and (peri-)urban dairy livestock systems: Opportunities

Milk is a highly perishable commodity. India doesn't have adequate transportation infrastructure to maintain the cold chain necessary to prolong the shelf life of animal-source foods, including milk. This has encouraged the creation of urban and (peri-)urban livestock markets in many cities in India, which has contributed to food security and met the demand for milk products in these areas (Prasad et al., 2019). Urban and (peri-)urban livestock can also fill a niche by making productive use of local food waste and by-products from agro-industries, thus reducing and recycling the organic waste of the cities (Schiere et al., 2001). However, milk and dairy product consumption is not the only reason to keep cattle in urban areas in India – female cattle also have both social and religious value as they are considered sacred in the Hindu faith. This religious value means that cattle are free to walk in crowded streets or through traffic, which may be a unique challenge and benefit in India.

4.4. Urban and (peri-)urban dairy livestock systems: Challenges

Despite the opportunities presented by urban and (peri-)urban livestock systems, they also face constraints as they can be a source of odor, disease and pollution. In urban and (peri-)urban livestock systems, there is specially a risk of zoonosis (diseases that affect both humans and animals, e.g. endoparasites) if the close cohabitation between humans and animals is combined with poor hygienic conditions on the farms. Thus, hygiene is of great importance for dairy cattle farming, especially in urban areas where the common cleaning practice is to flush the cattle excreta through the public drains. The inappropriate disposal of animal excreta contributes to the contamination of waterbodies (Prasad et al., 2019). Moreover, this inappropriate disposal can play a role in increasing the presence of insects and parasites, which contributes to food-borne diseases transmission, such as diarrhea (Delahoy et al., 2018). Furthermore, urban and (peri-)urban dairy systems are under great pressure due to the lack of space, labor shortage and

fodder scarcity, which can exacerbate animal health disorders (parasites, heat stress, mastitis and lameness) and can be detrimental to cow productivity, like milk yield, fertility and health.

4.5. Bangalore

Bangalore, officially known as Bengaluru, is the capital of the state of Karnataka, in Southern India. It is located on the Deccan plateau at 12.97° northern latitude, 77.59° eastern longitude and 920m above sea level. The climate is of tropical savannah type with two main periods: dry and humid. Bangalore was named the ‘Garden City’ in the early 20th century due to its numerous parks, gardens, lakes, abundant greenery and a pleasant climate throughout the year, which made it an ideal retirement city – a ‘Pensioners Paradise’ (Chacko, 2007; Sudhira et al., 2007). By the end of the 20th century, information technology (IT) companies began to establish themselves in Bangalore, also earning it the title of ‘the Silicon Valley of India’. With the development of the IT industry, other industries and sectors (e.g. textiles and biotechnology) also expanded, boosting Bangalore city's growth in economic, demographic and spatial terms.

Nowadays, Bangalore is one of the fastest growing cities in India (Figure 1.3); its area has grown seven times (Sudhira et al., 2007) and its population has grown nine times since 1965 (United Nations, Population Division, 2019c). According to the last census in 2011 (Directorate of census operations Karnataka, 2011), Bangalore had a population of 9.62 million, making it the third most populous city in India after Delhi and Mumbai, and it is expected to reach 16 million by 2021 (Groupe SCE India, 2016). Bangalore's population is mostly urban (90.9%), making it the most urbanized district in Karnataka (Directorate of census operations Karnataka, 2011).

With a growing urban population and a mostly vegetarian diet (Erlar, 2020), the dairy industry plays a crucial role in meeting the demand for milk and dairy products in the city of Bangalore. The concern over satisfying the increasing milk demand in urban areas was born in the 1960s, when programs were drawn up to develop the dairy industry. These programs had three objectives: (i) to increase milk production in rural areas; (ii) to improve the nutritional standard of the population; (iii) to strengthen the economy of the small and marginal farmers (Nyholm et al., 1974). Among the development programs for the dairy industry, "Operation Flood" stands out also known as the "White Revolution". It began in 1970 and laid the foundation for a dairy cooperative movement that linked village producers with urban consumers (Cunningham, 2009) which is still evident in Bangalore today (Lindahl et al., 2020). The establishment of

village dairy cooperatives has successfully integrated small and marginal farmers into Bangalore's changing food market.

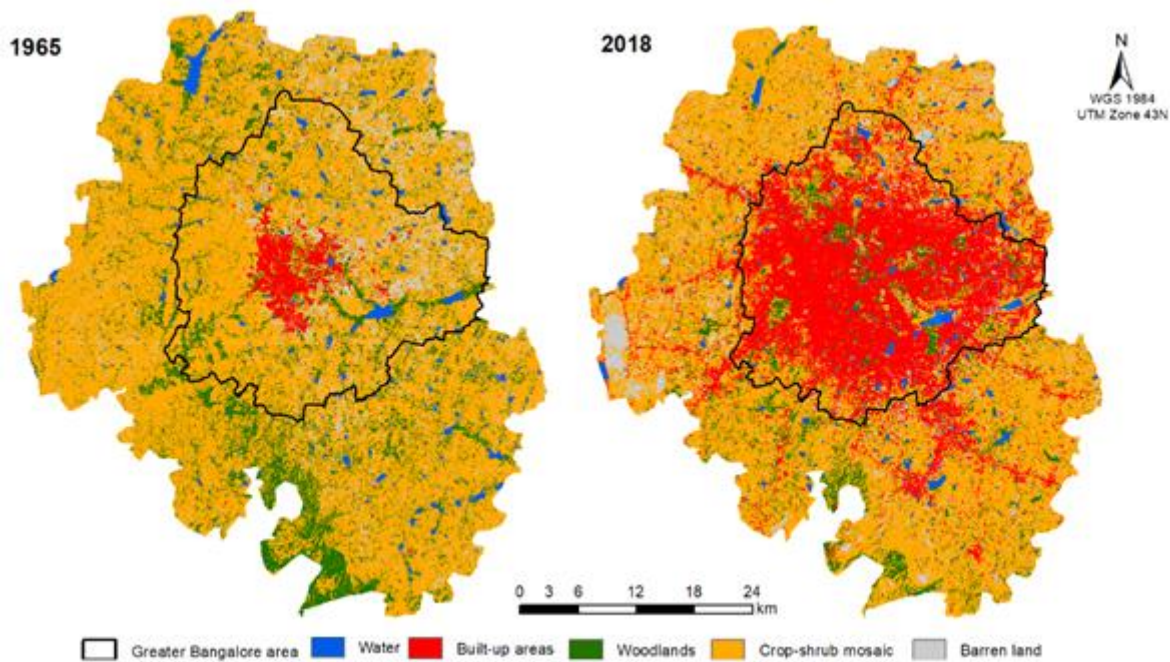


Figure 1.3. Land cover maps from 1965 and 2018 for the district Bangalore Urban. Adapted from Brinkmann et al. (2020).

4.5.1. Dairy livestock systems in Bangalore

The rapid urbanization, industrialization and the strong presence of dairy cooperatives managed through the Karnataka Milk Federation (KMF) has led to a steady increase of the dairy industry in Bangalore (Lindhahl et al., 2020; Prasad et al., 2019). The last livestock census in Bangalore shows a total lactating cow population of 160 thousand (National Dairy Development Board, 2015) which is a 14% increase from the previous census (Figure 1.4). This growth is mainly due to the increase of exotic dairy cattle and a decrease of native breeds especially in rural areas as shown in Figure 1.4. The most common exotic breed found in Bangalore is the Holstein Friesian followed by the Jersey cow. The most common native cow is the Hallikar, used mainly for draught purpose in rural areas (National Dairy Development Board, 2015). However, around 40% of cattle in the herds of Bangalore's farmers are crossbred, mixing exotic and native animals. Prasad (2019) defined, the urban and (peri-)urban dairy systems in Bangalore as semi-intensive systems; this included a herd size between 4 to 5 cattle and daily production between 10 to 20 kg of milk per head, the sale of which is primarily to neighbors and only secondarily to hotels, sweet shops or milk collection centers from KMF. Conversely, he defined rural dairy systems in Bangalore as low input-low output systems, since the average daily milk yield was only 8-10 kg per head, with a herd size between 3 to 5 cattle and the milk is mainly

sold to the dairy cooperative of the village. However, due to the considerably higher number of rural systems, the two systems combined contribute a similar amount (54% rural and 46% urban) to meet the demand for milk in Bangalore and are therefore both equally necessary.

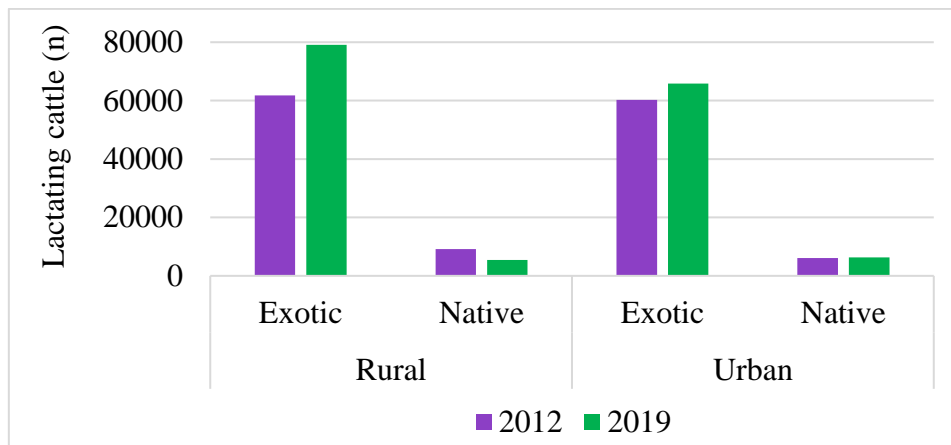


Figure 1.4. Number of exotic and native lactating cattle in rural and urban Bangalore during the last two Indian livestock census. (Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries, 2019, 2012).

5. Study objectives

This thesis, through the study of various dairy cattle traits (e.g. production traits, body condition, GHG emissions, cow wellbeing indicators and health traits) tries to unravel the effects of urbanization on dairy production. The focus is primarily on the social-ecological heterogeneity context of the megacity Bangalore though a rural-urban gradient (Figure 1.5).

1. In chapter 2 (A multivariate approach for the simulation of longitudinal dairy traits along continuous social-ecological gradients), the aim is to develop and evaluate a method for simulating true breeding values in a multiple-trait (milk yield and body condition) framework with relation to the continuous social-ecological gradient SSI, through random regression coefficients. Thus, allowing the reconstruction of missing covariances among traits or among random regression coefficients. Finally, it is applied to a derivation of economic weights on a daily "input-output" basis in the developed framework.

2. Chapter 3 (Phenotypic Dairy Cattle Trait Expressions in Dependency of Social-Ecological Characteristics along Rural–Urban Gradients) analyses different phenotypic dairy cattle traits (e.g. production traits, body condition, cow wellbeing indicators and health traits) via linear mixed modelling; it considers both social and ecological continuous descriptors simultaneously along a rural-urban gradient, choosing the megacity of Bangalore for its challenging environmental conditions and social complexity.

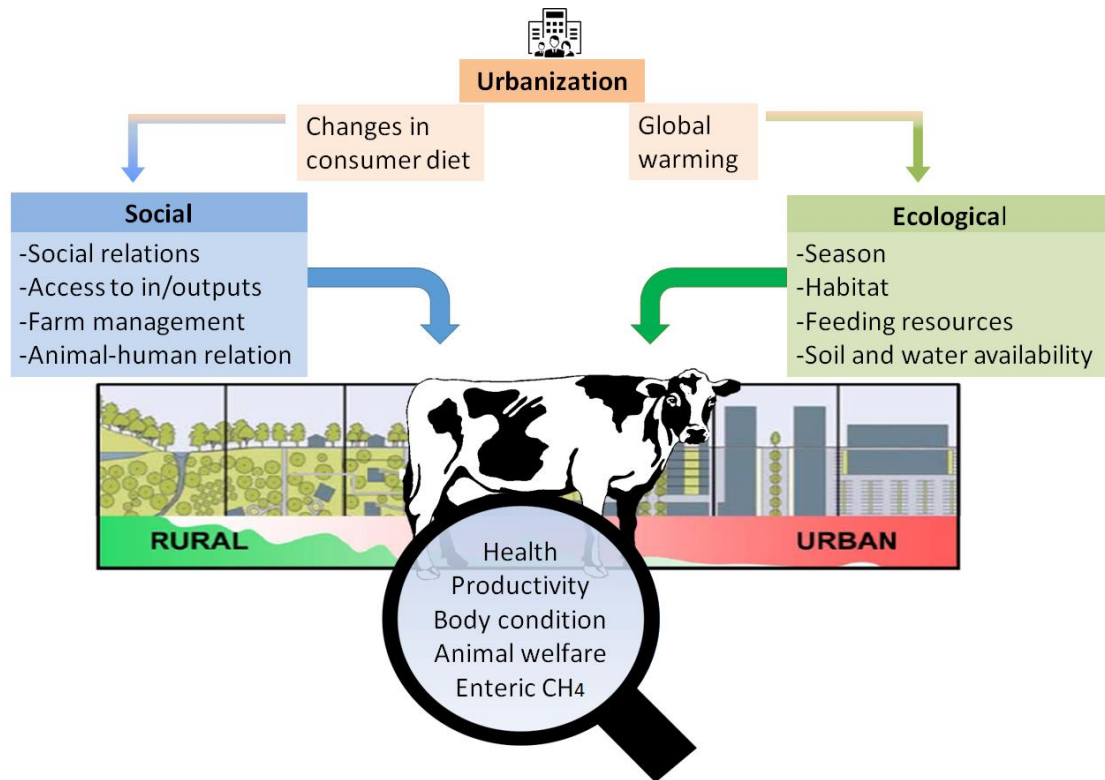


Figure 1.5. Conceptual framework depicting the social-ecological components of urbanization through a rural-urban gradient by analyzing dairy cattle traits.

3. The objective of chapter 4 (Enteric methane emission of dairy cattle along a rural-urban gradient in the Indian megacity Bangalore) is to evaluate the effects of social-ecological components on enteric CH₄ emissions exhaled by dairy cows measured through a portable laser methane detector, considering differences in dairy cattle herd management along a rural to urban gradient in Bangalore, India.

4. Chapter 5 (Gastrointestinal nematode and *Eimeria* spp. infections in dairy cattle along a rural-urban gradient) focuses on determining the prevalence of two gastrointestinal parasites – namely Gastrointestinal nematode (GIN) and *Eimeria* spp. – in dairy cattle along a rural to urban gradient in the Indian megacity of Bangalore. It also examines the effect of social-ecological components on the prevalence of and egg/oocyst counts of these gastrointestinal parasites along this gradient.

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CHAPTER 2

A Multivariate Approach for the Simulation of Longitudinal Dairy Traits along Continuous Social-Ecological Gradients

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1. Introduction

Dairy cattle, especially high yielding Holstein Friesian (HF) cows, react very sensitive to alterations on environmental scales. Environmental sensitivity especially includes challenges due to heat stress (Brügemann et al., 2012), heterogeneous grassland conditions (Jaeger et al., 2018), but also suboptimal indoor feeding and management conditions (König et al., 2005a). In these studies, environmental sensitive was defined as the decline in productivity and fertility in harsh environments. Also for disease traits, the individual farm management and human-animal interactions explained substantial differences in disease incidences and cow behavior pattern across herds (König et al., 2005b; Ebinghaus 2018). Both components, i.e. the human-animal relationships reflecting social characteristics and the classical environmental conditions (e.g. climate, feeding resources), were considered when defining social-ecological systems for livestock classifications (Martin-Collado et al., 2014).

Accordingly, Jaeger et al. (2016) identified pronounced genotype by environment interactions when considering simultaneously environmental and social classification criteria for herd grouping. Most obvious social-ecological challenges for dairy production due to obvious social-ecological heterogeneity might exist in rising megacities along rural-urban gradients. Schlesinger (2013) proposed the calculation of an urban-rural index (URI), combining building density and the road network as spatial characteristics on a scale from 0 (rural) to 1 (urban). The URI concept as developed by Schlesinger (2013) was the basis to classify the megacity Bangalore in India into “urban” (URI: 0.7 – 0.6), “(peri-)urban” (URI: 0.5 – 0.4) and “rural” (URI: < 0.3). Major farm differences on the URI-scale in Bangalore from a dairy perspective are related to herd size, livestock composition, milk production per household, and the amount of concentrates in the feeding ration. Recently, Hoffmann et al. (2017) developed a new survey stratification index (SSI), being specific for the rural-urban gradient in Bangalore. The SSI based on build-up density and distance to the city center on a scale from 1 to 6 in increments of 1 (1 = urban, 6 = rural).

Jaeger et al. (2016) not only identified substantial differences in phenotypic trait expressions in different social-ecological environments, but also heterogeneity for genetic (co)variance components, for HF as well as for local native dual-purpose breeds. In conclusion, they suggested the estimation of bull breeding values via multiple trait models. A multiple trait model application allows the estimation of specific bull breeding values for distinct environments, e.g. a specific breeding value for the same sire A for a grassland system, for an indoor system, etc. The international genetic evaluation system for dairy sires reflects such

ideas, but uses strict country borders instead of environmental classifications. A further enhancement in this regard is the so-called “borderless-clustering” methodology (Weigel and Rekaya, 2000), which is considering several environmental descriptors simultaneously.

However, in addition to environment-specific breeding values, overall breeding indices might differ on the social-environmental gradient. Breeding indices combine several traits of interest via economic weights. The challenge is to derive economic weights for the increasing number of breeding goal traits, and to consider environmental particularities. Dekkers and Gibson (1998) evaluated methods, which were proposed for the definition of breeding objectives, and for the derivation of economic weights. In most cases, profit functions were applied. In a dairy cattle breeding sense, “profit” is the net return or income after accounting for the expenditures related to a cow. Goddard (1998) recommended recording of net returns on an individual animal basis, in order to circumvent the definition of a specific profit function and to avoid complex bio-economic modelling. St-Onge et al. (2002) took data from Quebec herds and calculated economic values for different traits by regressing net returns on estimated breeding values. However, information about all traits, diseases, management practices and costs are generally not available to determine the net returns per cow per day of herd life. As an alternative, a very detailed stochastic simulation of cows in a herd could determine net returns per cow and day.

Random regression methodology allows the estimation of genetic parameters on continuous scales (time scale, but also environmental scales), and the simulation of precise dairy cow records in different environments (Schaeffer, 2004). On a biological basis, there could be different genes that turn on and off with progressing time (e.g. days in milk, aging), but also with obvious environmental changes as experimentally proven in heat stressed male mice (Cammack et al., 2009). A classic example from official genetic evaluations addresses test-day model applications for production traits. Here, random regression models can account for the differences in means and variances of milk production during lactation. Similarly, Al-Kanaan et al. (2015) optimized genetic evaluations in dependency of continuous temperature x humidity indices via random regression applications. Especially diseases have different incidences in different environments, with impact of disease frequencies on genetic (co)variance components.

Multivariate simulations in a random regression framework allow consideration of trait interactions via covariances among random regression coefficients. Multiple trait random regression models consider genetic, permanent environmental and residual correlations among traits. The advantages of multiple trait random regression models over single trait random

regression models increase with increasing heritability differences, and with increasing differences among pairwise correlations. Furthermore, a simulation in a multiple trait framework generates breeding values for traits which cannot be recorded for distinct animals, e.g. due to culling or lacking recording schemes. Hence, a detailed simulation should consider, and if missing, generate all breeding values and all phenotypic records for all cows. The number of traits to be considered are extremely large in existing dairy cattle breeding programs and will increase continuously due to the broadening of breeding goals (Miglior et al., 2005), but also due to a broader variety of social-ecological systems for dairy cattle husbandry. In random regression simulations, elements in (co)variance matrices strongly depend on the structure of covariance functions for longitudinal data. In this regard, Legendre polynomials allow a large extent of flexibility (Courant and Hilbert, 1953). For example, if genetic-statistical modelling utilizes Legendre polynomials of order 4, the (co)variance matrix of random regression coefficients for additive-genetic effects is of dimension 5 x 5 for a single trait. Simultaneous consideration of all traits in a multivariate approach requires setting up additive genetic, permanent environmental, and residual (co)variance matrices for all combinations of random regression coefficients. However, studies with a focus on the estimation of random regression coefficients in a multivariate approach considering the whole variety of production and functional traits are not available, or not feasible due to practical or statistical limitations.

Consequently, the aim of the present study was to develop a method to generate random regression coefficients in a multivariate approach for missing covariances among traits. In a first step, we explain the method in detail using fictitious data for different scenarios and restrictions. In a second step, for methodological evaluations, solutions for random regression coefficients for production traits are combined with random regression coefficients for body condition score. In a third step, we use random regression coefficients for additive-genetic effects on the continuous social-ecological gradient SSI, and combine animal solutions with fixed SSI-curves. In a fourth and final step, we introduce our idea for the derivation of economic weights on a daily “input-output basis” in the developed framework.

2. Materials and Methods

2.1 Explanation of the simulation

2.1.1. Simulation of true breeding values using fictitious data.

The method is illustrated for a multivariate problem including 6 traits. Assume an arbitrary matrix \mathbf{G} of additive-genetic (co)variances such as:

$$G = \begin{bmatrix} 25 & 5 & -10 & 10 & -5 & 5 \\ 5 & 50 & 5 & -5 & 13 & -13 \\ -10 & 5 & 86 & -5 & 31 & 5 \\ 10 & -5 & -5 & 30 & -9 & 9 \\ -5 & 13 & 31 & -9 & 31 & -7 \\ 5 & -13 & 5 & 9 & -7 & 17 \end{bmatrix}$$

The general steps for generating true breeding values (TBV) are as follows:

1. Do Cholesky-decomposition of \mathbf{G} resulting in:

$$\mathbf{L}_G = \begin{bmatrix} 5 & 0 & 0 & 0 & 0 & 0 \\ 1 & 7 & 0 & 0 & 0 & 0 \\ -2 & 1 & 9 & 0 & 0 & 0 \\ 2 & -1 & 0 & 5 & 0 & 0 \\ -1 & 2 & 3 & -1 & 4 & 0 \\ 1 & -2 & 1 & 1 & -1 & 3 \end{bmatrix}$$

2. Generate a vector \mathbf{v} of 6 random normal deviates, e.g.:

$$\mathbf{v} = \begin{bmatrix} -0.426 \\ 0.112 \\ -1.698 \\ -1.691 \\ 1.038 \\ -0.107 \end{bmatrix}$$

3. Generate TBV for one animal in vector \mathbf{w} via multiplying matrix \mathbf{L}_G with vector \mathbf{v} :

$$\mathbf{w} = \begin{bmatrix} -2.134 \\ 0.357 \\ -14.324 \\ -9.419 \\ 1.399 \\ -5.401 \end{bmatrix}$$

Generation of a single covariance matrix for missing covariances among traits. Consider the Matrix \mathbf{G} as described above, but divided into 2 sub-matrices \mathbf{G}_1 and \mathbf{G}_2 . The common overlapping trait with variance 86 in both matrices is trait 3 in \mathbf{G}_1 and trait 1 in \mathbf{G}_2 .

$$\mathbf{G}_1 = \begin{bmatrix} 25 & 5 & -10 \\ 5 & 50 & 5 \\ -10 & 5 & 86 \end{bmatrix} \text{ and } \mathbf{G}_2 = \begin{bmatrix} 86 & -5 & 31 & 5 \\ -5 & 30 & -9 & 9 \\ 31 & -9 & 31 & -7 \\ 5 & 9 & -7 & 17 \end{bmatrix}$$

The general steps for generating a single \mathbf{G} matrix containing covariances among all traits are:

1. Do Cholesky-decomposition of \mathbf{G}_1 and \mathbf{G}_2 resulting in:

$$\mathbf{L}_{\mathbf{G}_1} = \begin{bmatrix} 5 & 0 & 0 \\ 1 & 7 & 0 \\ -2 & 1 & 9 \end{bmatrix} \text{ and } \mathbf{L}_{\mathbf{G}_2} = \begin{bmatrix} 9.274 & 0 & 0 & 0 \\ -0.539 & 5.451 & 0 & 0 \\ 3.343 & -1.321 & 4.252 & 0 \\ 0.539 & 1.704 & -1.541 & 3.381 \end{bmatrix}$$

2. Generate a vector \mathbf{v}_1 with 3 elements of random normal deviates, e.g.

$$\mathbf{v}_1 = \begin{bmatrix} -0.173 \\ 0.201 \\ 0.183 \end{bmatrix}$$

3. Generate TBV in vector \mathbf{w}_1 for one animal for the traits 1, 2, and 3 through the multiplication of matrix $\mathbf{L}_{\mathbf{G}_1}$ and vector \mathbf{v}_1 :

$$\mathbf{w}_1 = \begin{bmatrix} -0.867 \\ 1.238 \\ 2.193 \end{bmatrix}$$

4. Generate a vector \mathbf{v}_2 with 4 elements of random normal deviates, e.g.

$$\mathbf{v}_2 = \begin{bmatrix} -0.887 \\ -0.519 \\ -0.209 \\ -0.924 \end{bmatrix}$$

5. Replace the first element of vector \mathbf{v}_2 (here: value = -0.887) with the quotient x of the corresponding normal random deviate for the TBV from the overlapping trait in vector \mathbf{w}_1 (here: value = 2.193) and the element [1,1] of matrix $\mathbf{L}_{\mathbf{G}_2}$ (here: value = 9.274). Hence, the value for the quotient in the current example is $x = \frac{2.193}{9.274} = 0.236$. The modified vector \mathbf{v}_2 is:

$$\mathbf{v}_2 = \begin{bmatrix} 0.236 \\ -0.519 \\ -0.209 \\ -0.924 \end{bmatrix}$$

6. Generate TBV in vector \mathbf{w}_2 for one animal for the traits 3, 4, 5, and 6 through the multiplication of matrix \mathbf{L}_{G_2} and vector \mathbf{v}_2 :

$$\mathbf{w}_2 = \begin{bmatrix} 2.189 \\ -2.954 \\ 0.585 \\ -3.560 \end{bmatrix}$$

7. Drop the first element of vector \mathbf{w}_2 and combine elements of vector \mathbf{w}_1 and vector \mathbf{w}_2 in a single-column vector \mathbf{z} :

$$\mathbf{z} = \begin{bmatrix} -0.867 \\ 1.238 \\ 2.193 \\ -2.954 \\ 0.585 \\ -3.560 \end{bmatrix}$$

8. Generate a matrix $\mathbf{P} = \mathbf{z}\mathbf{z}'$:

$$\mathbf{P} = \begin{bmatrix} 0.752 & -1.073 & -1.901 & 2.561 & -0.507 & 3.087 \\ & 1.533 & 2.715 & -3.657 & 0.724 & -4.407 \\ & & 4.809 & -6.478 & 1.283 & -7.807 \\ & & & 8.726 & -1.728 & 10.516 \\ & & & & 0.342 & -2.083 \\ \text{sym.} & & & & & 12.673 \end{bmatrix}$$

9. Perform steps 2 through 8 in a loop of n replicates. Save all \mathbf{P} -matrices, and create matrix \mathbf{H} by summing up all \mathbf{P} matrices.

10. Save the elements of the first row of matrix \mathbf{H} in Vector \mathbf{d} .

11. Create the final matrix \mathbf{Z} , i.e. correct \mathbf{H} for the degrees of freedom as follows:

$$\mathbf{Z} = \frac{(\mathbf{H} - (\mathbf{d}\mathbf{d}')) * \frac{1}{n}}{n-1}$$

2.1.2 Methodological evaluation for random regression coefficients

Databases were random regression coefficients for additive-genetic and permanent environmental effects from the six traits milk-kg, protein-kg, fat-kg, lactose, somatic cell score (SCS), and milk urea nitrogen (MUN) (dataset A). (Co)variance components among random regression coefficients for these traits were estimated as described in a study by Miglior et al. (2007). The modelling of regression curves based on the approach by Jamrozik et al. (2002), who used Legendre polynomials of order 4 for additive-genetic and permanent environmental effects in dependency of days in milk.

Consequently, (co)variance matrices of both additive-genetic and permanent environmental effects were of dimension 90 x 90, including 5 coefficients per trait and lactation (6 traits x 5 coefficients x 3 lactations). Matrices were half-stored and sorted by regression coefficients (intercept to Legendre of order 4) within lactations (1 to 3) and within traits (milk-kg, protein-kg, fat-kg, lactose, SCS, MUN). To give an example, element [1,1] refers to the variance of the intercept for milk yield in first lactation, and element [90,90] is the fourth random regression coefficient of polynomial 4 for MUN in parity 3. The whole matrix was divided into different sub-matrices of different sizes, in order to evaluate our method for different scenarios. A particularity of a random regression model application compared to a “classical” multiple-trait model is the following: A simulation study based on random-regression coefficients has in general more than one common overlapping element. Hence, the extent of overlapping depends on the defined covariance structure and on the number of common traits in sub-matrices \mathbf{G}_1 and \mathbf{G}_2 . The strategy for creating sub-matrices \mathbf{G}_1 and \mathbf{G}_2 with different dimensions and different numbers of overlapping elements from the known matrix \mathbf{G} is explained in Figure 2.1.

The applied strategy allows the reconstruction of the entire \mathbf{G} -matrix for different scenarios of overlapping elements, as well as the construction of squared matrices with smaller dimensions than the original \mathbf{G} . The dimension of the reconstructed matrix \mathbf{Z} depends on the dimension of sub-matrix \mathbf{G}_1 (M_1) plus the dimension of sub-matrix \mathbf{G}_2 (M_2) minus the dimension of the overlapping square (M_3). Full reconstruction of the original \mathbf{G} of dimension 90 x 90 implies the inclusion of a starting point (element) $x_{ij} = [1,1]$ in \mathbf{G}_1 , and the inclusion of the last element $y_{ij} = [90,90]$ in \mathbf{G}_2 for a variable size of M_3 . Elements in the reconstructed \mathbf{Z} -matrix were compared with the corresponding elements in \mathbf{G} , using different statistical procedures.

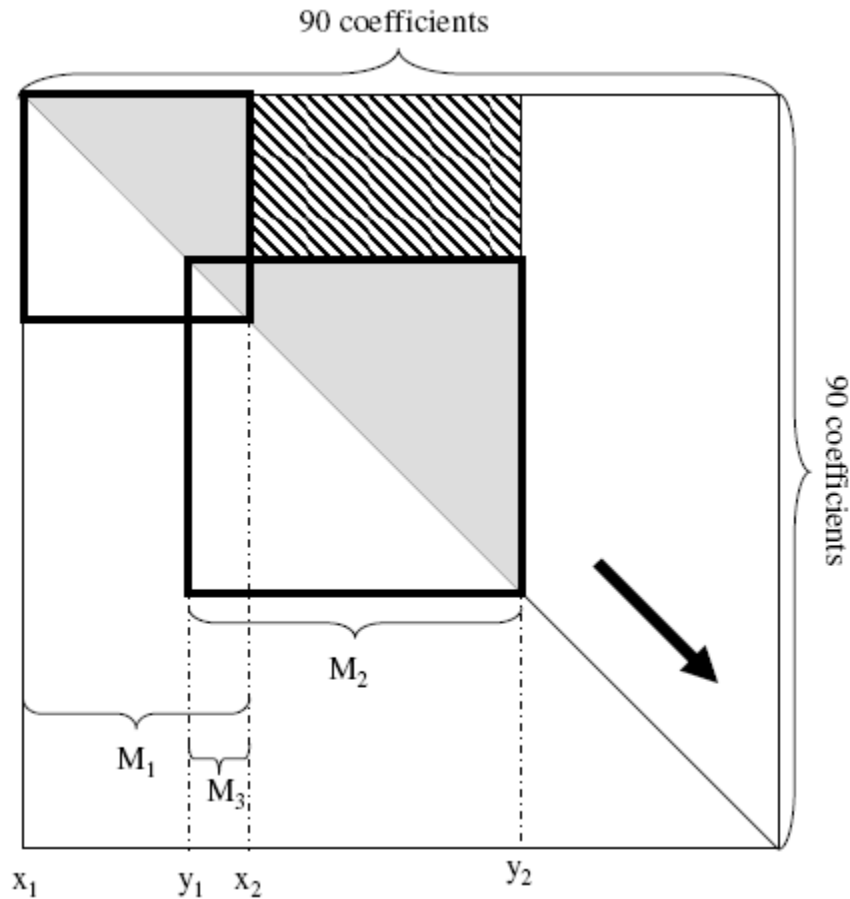


Figure 2.1. Procedure to create sub-matrices using random regression coefficients from Legendre polynomials of order 4 for milk-kg, fat-kg, protein-kg, SCS, and MUN from parities 1, 2, and 3 from a symmetric matrix \mathbf{G} of dimension 90 x 90. (x_1 = first element from sub-matrix \mathbf{G}_1 , x_2 = last element from sub-matrix \mathbf{G}_1 , y_1 = first element from sub-matrix \mathbf{G}_2 , y_2 = last element from sub-matrix \mathbf{G}_2 , M_1 = dimension of sub-matrix \mathbf{G}_1 , M_2 = dimension of sub-matrix \mathbf{G}_2 , M_3 = dimension of the overlapping square from matrices \mathbf{G}_1 and \mathbf{G}_2 , grey triangle = existing (co)variances among random regression coefficients, shaded rectangle = unknown covariances among random regression coefficients; arrow indicates the movement of sub-matrices for variable dimensions of M_1 and M_2 and different starting points x_1 and x_2).

2.1.3 Creation of phenotypic records on the rural-urban gradient

The fixed curve for milk-kg and BCS on the SSI-scale based on real data from 165 milking cows recorded in Bangalore from August 2016 until December 2017. BCS was recorded on a scale from 1 to 5, allowing increments of 1. The score 1 represented skinny cows, and score 5 indicated cows with obvious body fat depots. The BCS recording scheme is described by Ferguson et al. (1994). In order to generate the fixed curve in dependency of SSI, the following statistical model [1] was applied:

$$y_{ijklmn} = \text{herd}_i + \text{month}_j + \text{DIM}_k + \text{cow}_l + \text{SSI}_m + e_{ijklmn} \quad [1]$$

where y_{ijklmn} = test-day observations for milk-kg or BCS; month_j = fixed effect of the j -th month of trait recording; DIM_k = fixed effect of the k -th class for days in milk according to Huth

(1995); cow_1 = random effect of the l -th cow (for the repeated measurements); SSI_m = covariate stratification index; and e_{ijklmn} = random residual component. The continuous social-environmental descriptor SSI was generated with Legendre polynomials 1, 2, 3 or 4 in consecutive runs. Legendre polynomials were standardized in the range from 0 (urban) to 1 (rural).

Simulation of phenotypic cow records for milk-kg and BCS along the social-ecological gradient in Bangalore was done on the basis of the generated fixed SSI-curve, combined with the random regression coefficients from the meta analyses. Meta analyses means inclusion of the random regression coefficients for production traits from the previously introduced six traits (dataset A). The random regression coefficient matrix for production traits was combined with random regression coefficients for milk-kg and BCS in first lactation (dataset B) from the study by Veerkamp et al. (2001). For BCS, too, the random regression model based on Legendre polynomials of order 4. Matrix \mathbf{G}_2 was of dimension 10 x 10 including the (co)variance components of random regression coefficients of milk-kg and BCS from first lactation. Matrix \mathbf{G}_1 was the entire matrix of (co)variance components from dataset A of dimension 90 x 90. The overlap square contained milk-kg from lactation 1. All (co)variances among random regression coefficients for milk-kg were from dataset A, in matrix \mathbf{G}_1 as well as in matrix \mathbf{G}_2 . (Co)variances for random regression coefficients for BCS and covariances among random regression coefficients for milk-kg and BCS were used from dataset B. In a next step, we extracted the random regression coefficients for additive-genetic and permanent environmental effects for milk-kg and BCS from the constructed \mathbf{Z} -matrix, resulting in a \mathbf{Z}_1 -submatrix. The \mathbf{Z}_1 -submatrix was the basis to simulate phenotypic observations and breeding values on the SSI scale (again: standardized SSI scale from 0 = urban to 1 = rural).

The random regression model [2] for simulating phenotypic observations for the two traits milk-kg and BCS was:

$$\begin{pmatrix} y_{1j} \\ y_{2j} \\ \dots \\ y_{nj} \end{pmatrix} = \begin{pmatrix} FC(1) \\ FC(2) \\ \dots \\ FC(n) \end{pmatrix} + \begin{pmatrix} PA(1)_j \\ PA(2)_j \\ \dots \\ PA(n)_j \end{pmatrix} + (b_{ii})^{-5} \mathbf{L}_G \begin{pmatrix} RND(1) \\ RND(2) \\ \dots \\ RND(k) \end{pmatrix} \dots_{jk} + \mathbf{L}_{PE} \begin{pmatrix} RND(1) \\ RND(2) \\ \dots \\ RND(k) \end{pmatrix} \dots_{jk} + \mathbf{L}_R \begin{pmatrix} RND(1) \\ RND(2) \\ \dots \\ RND(k) \end{pmatrix} \dots_{jk} \quad [2]$$

where y is the phenotypic observation, FC is the respective fixed SSI-curve, and PA is the respective parent average for one of the n -traits at SSI j . Cholesky-decomposition was applied to obtain the lower triangular matrices \mathbf{L}_G , \mathbf{L}_{PE} , and \mathbf{L}_R such that:

$$\mathbf{G} = \mathbf{L}_G \mathbf{L}'_G$$

$$\mathbf{PE} = \mathbf{L}_{PE} \mathbf{L}'_{PE}$$

$$\mathbf{R} = \mathbf{L}_R \mathbf{L}'_R$$

where \mathbf{G} , \mathbf{PE} , and \mathbf{R} are the covariance matrices of random regression coefficients for additive-genetic, permanent environmental, and residual effects, respectively. Pseudo-random normal deviates (RND) with $\mu = 0$ and $\sigma = 1$ were assigned to all random regression coefficients k . Matrix $\Phi = \mathbf{M} \Lambda$ with Λ being the matrix of Legendre polynomials, and \mathbf{M} being a matrix containing the polynomials of standardized SSI values in the range from 0 to 1. Elements b_{ii} are from matrix \mathbf{B}^5 , which is a diagonal matrix containing the diagonal elements of matrix \mathbf{T} . Any positive definite, symmetric matrix such as the additive-genetic relationship matrix \mathbf{A} can be written as the product of a matrix \mathbf{T} times its transpose \mathbf{T}' (Quaas, 1976; Meuwissen and Luo, 1992). In the simulation, we also considered the fixed effects as described in model [1], allowing the modelling of phenotypic records for specific combinations of continuous SSI values and classes of fixed effects, e.g. days in milk.

3. Results and Discussion

3.1. Results from a fictitious (co)variance matrix including 6 traits

We evaluated the proposed method for different numbers of replicates (i.e. for 10, 100, 1,000, 10,000, and 100,000 replicates, named \mathbf{Z}_{10} , \mathbf{Z}_{100} , \mathbf{Z}_{1000} , \mathbf{Z}_{10000} , \mathbf{Z}_{100000} , respectively) using the fictitious data in matrix \mathbf{G} . The comparison of elements of different G-matrices is summarized in Table 2.1 for the diagonal elements (= variances), in Table 2.2 for the existing covariances among traits, and in Table 2.3 for the unknown covariances among traits. Simulated variances were completely identical compared to the elements from original \mathbf{G} -matrix, when considering 10,000 replicates, and when using the average of all 10,000 matrices for computing elements in \mathbf{Z}_{10000} . Consequently, \mathbf{Z}_{100000} gave same results as shown in \mathbf{G} and \mathbf{Z}_{10000} . When comparing existing covariances among traits for different numbers of replicates, only the element in sub-cell [1,3] (and also the corresponding element [3,1] of the symmetric matrix) was slightly different for 10,000 replicates (\mathbf{Z}_{10000}) and 100,000 replicates (\mathbf{Z}_{100000}), i.e. 11 *versus* 10. The latter value corresponds to the value in \mathbf{G} . Minor differences were found for the unknown covariances, even for 100,000 replicates. However, the unknown covariances in $\mathbf{Z}_{1000000}$ have the same algebraic sign as the original values. When depicting the future trend in a complex breeding goal comprising production, conformation, functionality, and health, the proper algebraic sign of correlations or covariances among traits is the crucial point. Application of a multivariate approach usually needs some modification of existing parameters, e.g. procedures

to make a matrix positive definite for Cholesky-decomposition. Nevertheless, due to inconsistencies of covariances in Table 2.3 for low numbers of replicates, we suggest at least 100,000 replicates for a similar problem with six traits, two-submatrices and one overlapping element.

Table 2.1. Comparison of diagonal elements of \mathbf{Z} -matrices for different numbers of replicates with corresponding elements of matrix \mathbf{G} (original fictitious covariance matrices for 6 traits).

Matrix	No. of replicates	Element in matrix \mathbf{G}					
		[1,1]	[2,2]	[3,3]	[4,4]	[5,5]	[6,6]
\mathbf{G}	Original matrix	25	50	86	30	31	17
\mathbf{Z}_{10}	10	17	35	40	24	22	30
\mathbf{Z}_{100}	100	27	37	89	30	28	18
\mathbf{Z}_{1000}	1,000	26	46	87	28	30	18
\mathbf{Z}_{10000}	10,000	25	50	86	30	31	17
\mathbf{Z}_{100000}	100,000	25	50	86	30	31	17

Table 2.2. Comparison of existing covariance elements of \mathbf{Z} -matrices for different numbers of replicates with corresponding elements of matrix \mathbf{G} (original fictitious covariance matrices for 6 traits).

Matrix	No. of replicates	Element in matrix \mathbf{G}								
		[1,2]	[1,3]	[2,3]	[3,4]	[3,5]	[3,6]	[4,5]	[4,6]	[5,6]
\mathbf{G}	Original matrix	5	-10	5	-5	31	5	-9	9	-7
\mathbf{Z}_{10}	10	12	-14	-7	20	16	16	-2	8	-2
\mathbf{Z}_{100}	100	4	-9	14	-5	30	8	-8	9	-5
\mathbf{Z}_{1000}	1,000	6	-10	5	-4	30	7	-7	9	-7
\mathbf{Z}_{10000}	10,000	5	-11	4	-4	30	5	-9	9	-7
\mathbf{Z}_{100000}	100,000	5	-10	5	-5	31	5	-9	9	-7

Table 2.3. Comparison of unknown covariance elements of \mathbf{Z} -matrices for different numbers of replicates with corresponding elements of matrix \mathbf{G} (original fictitious covariance matrices for 6 traits).

Matrix	No. of replicates	Element in matrix \mathbf{G}					
		[1,4]	[1,5]	[1,6]	[2,4]	[2,5]	[2,6]
\mathbf{G}	Original matrix	10	-5	5	-5	13	-13
\mathbf{Z}_{10}	10	-14	-3	-5	-12	5	-13
\mathbf{Z}_{100}	100	-2	-5	-1	-6	8	0
\mathbf{Z}_{1000}	1,000	1	-4	-1	1	2	2
\mathbf{Z}_{10000}	10,000	0	-4	0	0	2	-7
\mathbf{Z}_{100000}	100,000	1	-4	1	-1	2	-7

3.2. Results from a (co)variance matrix for existing random regression coefficients

In general, practical situations are more complex, i.e. characterized by a larger number of traits and more than one overlapping element. Evaluation of our methodology was done for different scenarios using the random regression coefficients for additive-genetic and permanent environmental effects from dataset A as illustrated in Figure 2.1. The first focus was on the additive-genetic coefficients and on the re-construction of the entire original 90 x 90 \mathbf{G} -matrix (defined as matrix \mathbf{Z} in the methodology) for different numbers of overlapping elements, implying variation of the size parameter M_3 by extending matrix \mathbf{G}_1 . Hence, \mathbf{G}_1 comprised 10 random regression coefficients for milk-kg from lactation 1 and 2 in the first scenario. In subsequent scenarios, we extended \mathbf{G}_1 through stepwise inclusion of random regression coefficients for milk-kg from lactation 3, and fat-kg, SCS, and lactose from all lactations. Matrix \mathbf{G}_2 was fixed to dimension 85 x 85 including random regression coefficients for milk-kg from lactation 2 and 3, as well as for all other traits and lactations. Table 2.4 shows equations of regression curves (determined by the intercept and the slope of regression) for an element-by-element comparison of the re-constructed \mathbf{Z} -matrix of dimension 90 x 90 with the original \mathbf{G} -matrix. In this regard, we compared a) all elements, b) the known coefficients in the grey triangle, and c) the unknown covariances to be estimated in the in shaded rectangle (as explained in Figure 2.1). Expectation values for intercept and slope were 0 and 1, respectively, resulting in identical \mathbf{Z} - and \mathbf{G} -matrices. For the most realistic scenario in practice, i.e. an overlapping of sub-matrices \mathbf{G}_1 and \mathbf{G}_2 through one shared trait (here: 5 shared regression coefficients for milk-kg in lactation 2, which implies $M_3 = 5$), parameters from the regression line reflected the expectations. Values were 0.001 for the intercept and 0.88 for the slope for all elements in the matrix, 0.001 for the slope and 0.99 for the intercept for the newly re-constructed, but already known elements, and 0.001 for the intercept and 0.49 for the slope for the unknown covariances (Table 2.4, first line). Almost identical values for the reconstructed elements in the \mathbf{Z} -matrix and in the original \mathbf{G} -matrix imply high coefficients of determination for the regression curve of 0.95, 0.98, and 0.87 for all elements, the existing elements, and the unknown elements, respectively. A larger dimension for overlapping coefficients in sub-matrices \mathbf{G}_1 and \mathbf{G}_2 , i.e. an increase of M_3 from 10 to 80, did not contribute to a more precise reconstruction of elements. This is due to a reshuffling of already known (co)variances by using random normal deviates affecting all (co)variances. The most practical scenario considering the parameters $x_1 = 1$, $M_1 = 10$, $x_2 = 6$, $M_2 = 85$, and $M_3 = 5$ generated very similar variances, and all covariances had the same sign. This is in agreement with the brief example for the arbitrary matrix of dimension 6 x 6 as illustrated in the example above.

Table 2.4. Comparison of reconstructed (co)variances and original (co)variances of additive-genetic random regression coefficients for matrix **G** of dimension 90 x 90 via linear regression for all elements, existing elements, and unknown elements (n = number of elements in **G**, int = intercept of regression line, b = slope of regression line, R² = coefficient of determination).

Parameters describing sub-matrices G_1 and G_2 ¹⁾					All elements (n = 4095, half stored)			Existing elements of a half-stored matrix				Unknown elements of a half-stored matrix			
x_1	M_1	x_2	M_2	M_3	int	b	R ²	N	Int	b	R ²	n	int	b	R ²
1	10	6	85	5	0.001	0.88	0.95	3695	0.001	0.99	0.98	400	0.001	0.49	0.87
1	15	6	85	10	0.001	0.96	0.94	3720	0.001	0.98	0.95	375	0.001	0.31	0.15
1	20	6	85	15	0.002	0.97	0.94	3745	0.002	0.98	0.95	350	0.002	0.26	0.13
1	25	6	85	20	0.002	0.97	0.94	3770	0.002	0.98	0.95	325	0.003	0.25	0.11
1	30	6	85	25	0.002	0.97	0.93	3795	0.002	0.98	0.94	300	0.002	0.29	0.13
1	35	6	85	30	0.002	0.98	0.93	3820	0.002	0.98	0.94	275	0.003	0.25	0.10
1	40	6	85	35	0.002	0.99	0.93	3845	0.002	0.99	0.94	250	0.003	0.18	0.08
1	45	6	85	40	0.002	0.98	0.93	3870	0.002	0.96	0.94	225	0.001	0.29	0.14
1	50	6	85	45	0.002	0.97	0.93	3895	0.002	0.97	0.94	200	0.002	0.30	0.17
1	55	6	85	50	0.002	0.96	0.81	3920	0.002	0.97	0.81	175	0.001	0.22	0.11
1	60	6	85	55	0.003	0.99	0.84	3945	0.003	0.99	0.85	150	0.002	0.16	0.09
1	65	6	85	60	0.010	1.04	0.30	3970	0.010	1.04	0.30	125	0.004	0.03	0.01
1	70	6	85	65	0.010	1.02	0.30	3995	0.010	1.02	0.30	100	0.001	1.13	0.42
1	75	6	85	70	0.010	0.99	0.23	4020	0.010	0.99	0.29	75	0.003	2.71	0.04
1	80	6	85	75	0.003	0.99	0.79	4045	0.003	0.99	0.79	50	0.002	2.24	0.06
1	85	6	85	80	0.001	0.98	0.95	4070	0.001	0.98	0.95	25	0.001	2.79	0.09

¹⁾Parameters as explained in Fig. 1

Table 2.5 depicts results for re-constructed **G**-matrices of different dimensions. Sub-matrix **G**₁ was of dimension 10 x 10 for all scenarios by including additive-genetic random regression coefficients for milk-kg from lactation 1 and 2. Sub-matrix **G**₂ was extended by the stepwise inclusion of traits, i.e. via the inclusion of random regression coefficients for milk-kg from lactations 1 and 2 in the first scenario, and all traits apart from milk-kg in first lactation in the last scenario. Hence, the reconstructed **Z**-matrix was of dimension 15 x 15 in the first, and of dimension 90 x 90 in the last scenario. The overlapping square was set up via five regression coefficients from milk-kg in lactation 2, implying a constant parameter M3 (Figure 2.1) for all scenarios. A smaller dimension of matrices **G**₂, also implying a smaller **Z**-matrix, was associated with a more precise reconstruction of elements. When comparing all elements in

matrices \mathbf{G} and \mathbf{Z} for a scenario with matrix \mathbf{G}_1 including random regression coefficients for milk-kg from lactations 1 and 2, and a matrix \mathbf{G}_2 only including random regression coefficients from milk-kg from lactation 2 (Table 2.5, first row), the intercept was 0.002, the slope was 0.58, and R^2 was 0.94. The element-by-element comparisons for variances of random regression coefficients of additive-genetic effects for this scenario are shown in Figure 2.2. The equation of the regression line with intercept = 0.002, slope = 1.02, and $R^2 = 0.99$ underlines the accurate reconstruction. Results for random regression coefficients of permanent environmental effects (not shown) followed the same pattern as shown for the additive-genetic random regression coefficients in Table 2.4 and Table 2.5.

Table 2.5. Comparison of reconstructed (co)variances and original (co)variances of additive-genetic random regression coefficients for matrix \mathbf{G} of variable dimension via linear regression for all elements, existing elements, and unknown elements (n = number of elements in \mathbf{G} , int = intercept of regression line, b = slope of regression line, R^2 = coefficient of determination; sub-matrix \mathbf{G}_1 included random regression coefficients for milk-kg from lactation 1 and 2 in all scenarios, and the overlapping square was of dimension 5×5 in all scenarios).

Parameter describing sub-matrix \mathbf{G}_2^1		All elements of a half-stored matrix				Existing elements of a half-stored matrix				Unknown elements of a half-stored matrix			
x_2	M_2	n	int	b	R^2	n	int	b	R^2	n	int	b	R^2
6	10	120	0.001	0.92	0.96	95	0.005	1.02	0.98	25	0.002	0.58	0.94
6	15	210	0.000	0.91	0.96	160	0.003	1.02	0.99	50	0.009	0.55	0.94
6	20	325	0.001	0.92	0.96	250	0.002	1.02	0.99	75	0.003	0.56	0.95
6	25	465	0.002	0.91	0.96	365	0.002	1.02	0.99	100	0.002	0.55	0.95
6	30	630	0.000	0.92	0.96	505	0.001	1.03	0.99	125	0.003	0.56	0.94
6	35	820	0.000	0.92	0.96	670	0.001	1.03	0.99	150	0.003	0.56	0.94
6	40	1035	0.000	0.93	0.96	860	0.001	1.03	0.99	175	0.001	0.56	0.95
6	45	1275	0.000	0.93	0.96	1075	0.000	1.04	0.99	200	0.001	0.56	0.95
6	50	1540	0.000	0.90	0.95	1315	0.000	1.02	0.99	225	0.003	0.50	0.88
6	55	1830	0.000	0.91	0.95	1580	0.001	1.02	0.99	250	0.001	0.51	0.89
6	60	2145	0.000	0.91	0.95	1870	0.000	1.02	0.99	275	0.002	0.50	0.88
6	65	2485	0.000	0.91	0.95	2185	0.001	1.02	0.98	300	0.001	0.51	0.88
6	70	2850	0.001	0.87	0.94	2525	0.002	0.98	0.98	325	0.001	0.47	0.87
6	75	3240	0.001	0.90	0.95	2890	0.001	1.01	0.98	350	0.000	0.50	0.88
6	80	3665	0.000	0.89	0.95	3280	0.001	1.01	0.98	375	0.000	0.50	0.89
6	85	4095	0.001	0.88	0.95	3695	0.001	0.99	0.98	400	0.001	0.49	0.87

¹Parameters as explained in Fig. 1

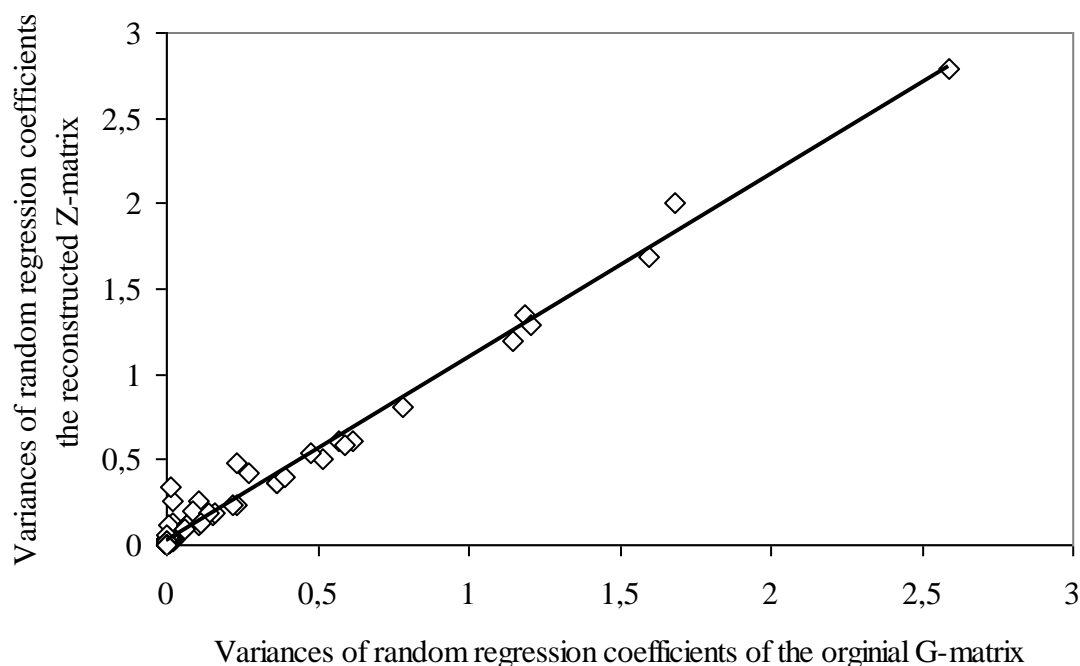


Figure 2.2. Comparison of variances for random regression coefficients for additive-genetic effects of a reconstructed \mathbf{Z} -matrix and the original \mathbf{G} -matrix (both of dimension 90×90), and the linear regression line (parameters of sub-matrices \mathbf{G}_1 : $x_1 = 1$, $x_2 = 10$ and \mathbf{G}_2 : $y_1 = 6$, $y_2 = 85$ as explained in Fig. 1).

3.3. Generating phenotypic trait curves in dependency of the rural-urban gradient

3.3.1 Solutions for fixed curves

Table 2.6 depicts the Akaike's information criterion (AIC; Akaike, 1973) and the Bayesian information criterion (BIC; Schwarz, 1978) for the modelling of SSI-curves. Low AIC- or BIC-values indicate model superiority. Hence, model quality improved when using Legendre polynomials of higher order, especially polynomials of order 4. Accordingly, superiority of Legendre polynomials over other covariance functions was identified for time dependent covariates in high-yielding Holstein cows (e.g. Gernand et al., 2013). Results suggest modelling of both, time or age curves, but also of environmental or social descriptor curves, with Legendre polynomials of order 3 or order 4. The generated fixed curve for milk yield in dependency of SSI (Figure 2.3) reflects the typical pattern of a lactation curve: An increase of daily milk yield from SSI 0 to 0.3 (comparable to the milk yield increase after calving up to the peak phase of lactation), and afterwards a slight decrease from SSI 0.3 to 1.0 (reflecting the curve pattern for the middle of lactation). For BCS (Figure 2.4), modelling fixed curves with Legendre polynomials of order 4 also depicts the curve pattern for BCS as known for European Holstein cows on the continuous time scale (i.e. in dependency of days in milk (e.g. Loker et al., 2011; Roche et al., 2009)). BCS decreased from SSI 0 to SSI 0.4 (being also the case on a time scale

after calving), had an intermediate maximum at SSI 0.62, and substantially decreased after SSI 0.85 (reflecting the end of lactation). When modelling the fixed SSI curve with only linear coefficients (Legendre polynomials of order 1), BCS slightly decreased on the continuous SSI scale (Figure 2.4). Almost identical SSI and lactation curves justify utilization of same random regression coefficients in the ongoing comprehensive simulation for both covariates days in milk and SSI.

Table 2.6. The Akaike information criterion (AIC) and the Bayesian information criterion (BIC) for the analyses of longitudinal test-day milk yield and body condition score from the statistical model [1] using different orders of Legendre polynomials for the covariate stratification index SSI (values in bold represent the lowest value for the respective evaluation criterion).

Trait and orders for Legendre polynomials	AIC	BIC
Milk yield		
Legendre 1	1781.48	1827.79
Legendre 2	1781.26	1824.64
Legendre 3	1780.73	1821.52
Legendre 4	1780.22	1818.08
Body condition score		
Legendre 1	378.14	428.29
Legendre 2	373.11	419.41
Legendre 3	371.28	413.71
Legendre 4	365.59	404.16

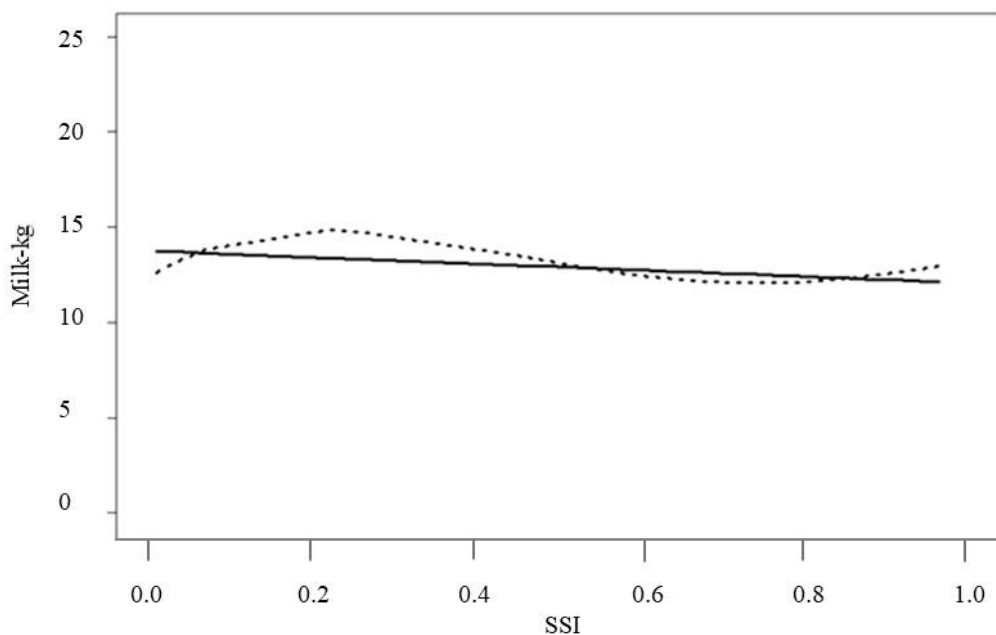


Figure 2.3. Solutions for fixed curves for daily milk yield (milk-kg) in dependency of the stratification index (SSI) in Bangalore, India. Dotted line: Modelling of the covariate SSI with Legendre polynomials of order 4; Solid line: Modelling of the covariate SSI with Legendre polynomials of order 1.

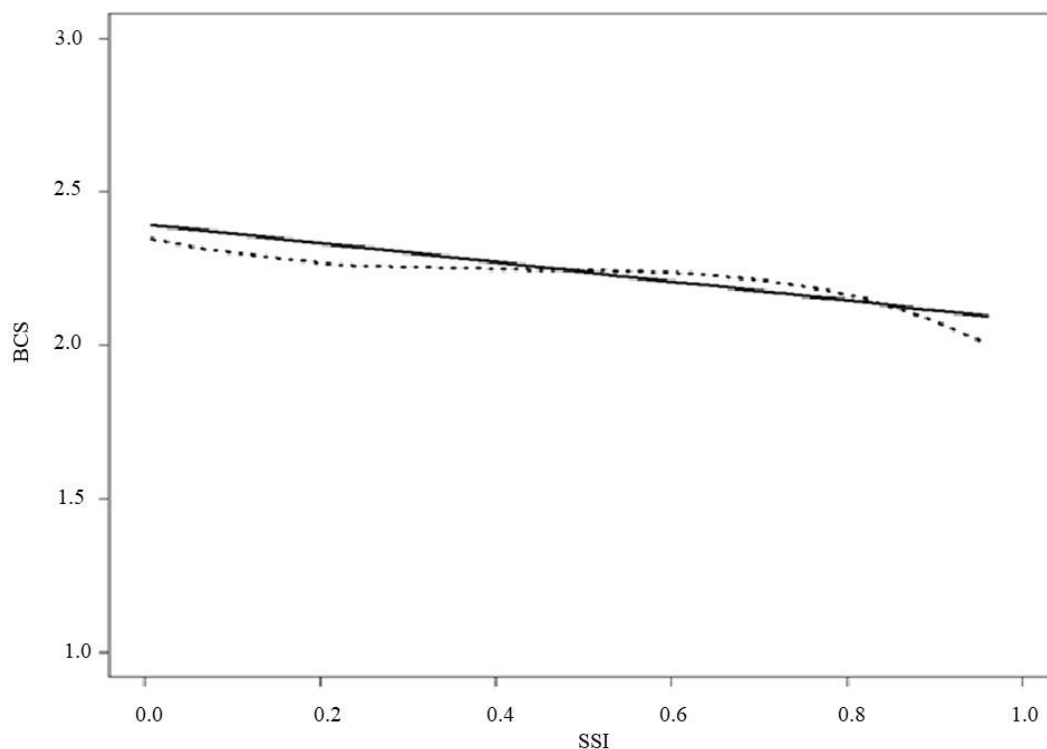


Figure 2.4. Solutions for fixed curves for body condition score (BCS) in dependency of the survey stratification index (SSI) in Bangalore, India. Dotted line: Modelling of the covariate SSI with Legendre polynomials of order 4; Solid line: Modelling of the covariate SSI with Legendre polynomials of order 1.

3.3.2 Simulation of phenotypic records along the rural-urban gradient

Solutions for fixed SSI-curves were combined with random regressions coefficients for additive-genetic and permanent environmental effects in model [2] in order to simulate phenotypic cow records for milk yield and BCS. The phenotypic curve pattern for milk-kg and for BCS in dependency of SSI reflected the pattern, which we expected from the raw phenotypic data. Both traits milk-kg (Figure 2.5) and BCS (Figure 2.6) are depicted in dependency of SSI for the early lactation stage, i.e. for the first Huth-class. Larger milk-kg combined with improved body shape (larger BCS values) for low SSI values reflect the improved dairy cattle management in the urban area in Bangalore (Pinto et al., 2018). However, we expect further model improvements and detailed validations of our methodology when using random regression coefficients directly estimated in Bangalore on the continuous SSI scale. Such an approach requires large datasets with a deep pedigree structure, or building up genomic relationship matrices via genomic marker data.

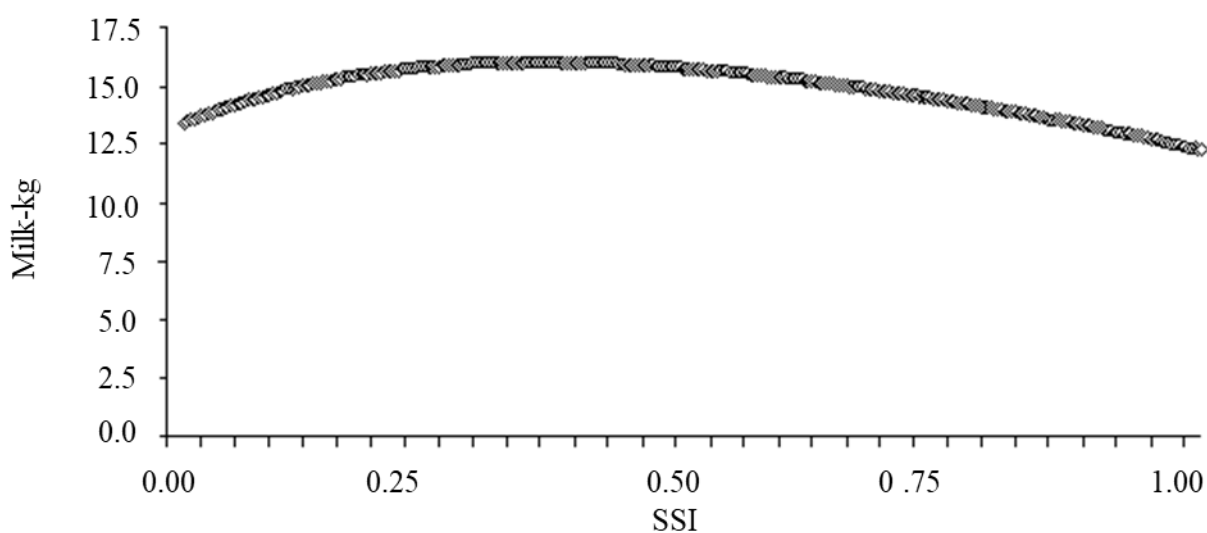


Figure 2.5. Phenotypic curve for milk-kg in dependency of SSI considering a cow from early lactation (Huth-class 1 (Huth, 1995)).

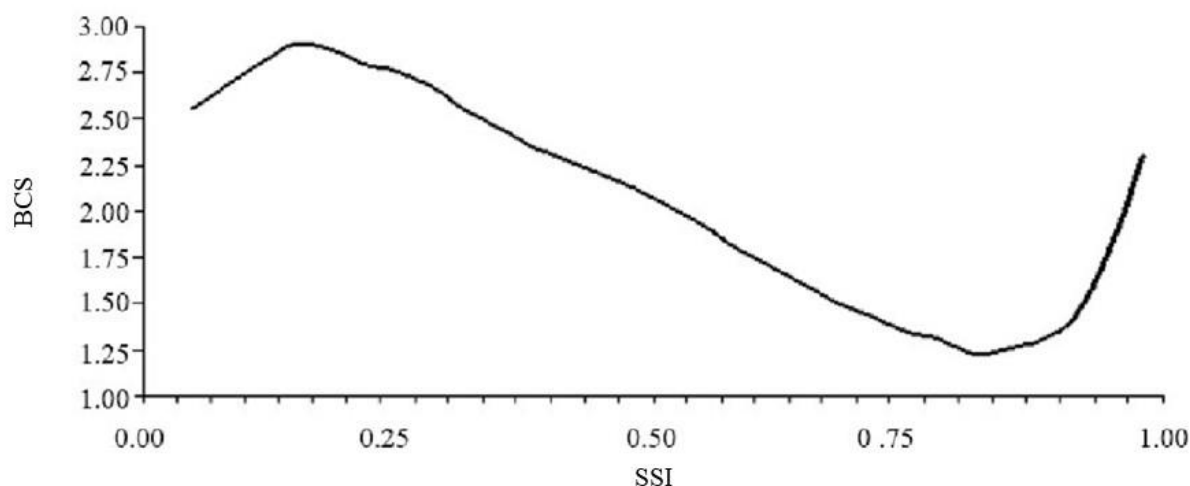


Figure 2.6. Phenotypic curve body condition score in dependency of SSI considering a cow from early lactation (Huth-class 1 (Huth, 1995)).

3.4 Perspective for the derivation of economic weights

The multivariate simulation approach with reconstructed covariances provides reliable phenotypic records, and allows the inclusion of a multitude of traits in a complex breeding goal. The idea also allows inclusion of further functional traits, especially health traits via random regression coefficients for “overlapping elements” from different studies. Milk yield was an appropriate link for creating overlapping squares in the present study, and BCS in the new \mathbf{Z} -matrix of dimension 95 x 95 generates connectedness to further functional trait categories, e.g. conformation traits (Wall et al., 2005), fertility (Veerkamp et al., 2001), and other body energy traits (Wall et al., 2007).

Such a comprehensive simulation simultaneously considering a large number of traits allows to mimic cow phenotypes on social-environmental gradients or continuous time scale. Also a random regression model including two covariates, i.e. URI or SSI combined with days in milk, could be applied as shown by Brügemann et al. (2011) for a combination of the covariates temperature x humidity index and days after calving. For each day x SSI combination, the amount of output variables of cows can be calculated. Major output variables are milk yield or protein yield. Multiplication of daily milk yield with milk prices allow the calculation of daily income, even for specific social-environmental farm types in order to account for possible genotype x environment interactions. On the other side, from the same cow, any health treatment due to a disease, or any unsuccessful insemination, are cost components on a daily basis. Daily income minus daily costs determine net returns per cow and day. With aging, daily net returns per cow can be accumulated, in order to calculate lifetime profit, or to calculate averaged net returns per cow and day. Simulation model [2] also generates true breeding values

from the same cows. In a multiple regression approach, averaged daily net returns (= the dependent variable) will be regressed on true breeding values for all traits being considered in the simulation. Hence, the regression coefficient is the economic weight for the trait of interest, i.e. the change in € per day for the increase of one trait unit for the true breeding value. However, such concept implies close collaboration with experts from economy, in order to collect all important revenues and expenses along days in milk and URI scales.

4. Conclusions

A precise simulation of daily dairy traits via multiple-trait models along social-ecological gradients imply availability of all covariance components among traits being considered in overall breeding goals or selection decisions. From a longitudinal trait recording perspective, covariances among random regression coefficients are required. The problem of missing covariances among traits or random regression coefficients can be solved when applying the idea as suggested in this study, i.e. basing on the general approach for simulating daily records in a multiple-trait framework. Methodology was evaluated via the comparison of genetic (co) variance components from created matrices with the original data. Furthermore, the methodology allowed the combination random regression coefficients from different studies due to “overlapping elements”, i.e. a subset of (co)variances among same traits considered in different studies. The framework was used to simulate daily cow records along the SSI. In this regard, we combined fixed curves for milk-kg and BCS in dependency of SSI (the phenotypic data recorded in Bangalore), with solutions for genetic (co)variance components of additive-genetic and permanent environment effects from meta analyses. Methodological evaluations, as well as the creation of phenotypic curves for milk-kg and BCS in dependency of SSI, resulted in reliable estimates. The individual variability of cows can be simulated simultaneously for a multitude of traits, and changes during life in production, growth, feed intake, and conformation traits can be modelled through random regression coefficients along social-ecological gradients. The basis for the methodology is presented in this study, and next steps to determine dairy cow efficiency should combine generated phenotypic records with input and output variables, i.e. returns and costs for specific days. Net returns per cow and day can be regressed on true breeding values, which are available from the simulation of additive-genetic random regression coefficients, in order to derive economic weights for breeding goal traits for specific social-ecological levels.

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CHAPTER 3

Phenotypic Dairy Cattle Trait Expressions in Dependency of Social-Ecological Characteristics along Rural–Urban Gradients

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1. Introduction

Traditional dairy cattle breeding focuses on improving milk yield or protein content, but with antagonistic impacts on functional traits such as fertility and health (Egger-Danner et al., 2015). However, high yielding breeds like Holstein Friesian (HF) are notably sensitive to environmental changes. In this regard, climate impacts due to global warming are a major current challenge for the dairy industry. Klinedinst et al. (1993) observed extended summer periods resulting in increased heat stress and detrimental effects on milk production and conception rate. Heat stress is also associated with reduced protein yield (Brügemann et al., 2011) and impaired female fertility (Wolfenson et al., 2000). In addition to summer season or heat stress impacts, management characteristics (König et al., 2005), feeding systems (Kolver et al., 2002), and heterogeneous grassland conditions (Jäger et al., 2016) are associated with phenotypic trait expressions in harsh environments. Social components such as farm management characteristics and human–animal relationships (as well as their interactions) also influence susceptibility to disease infections (Ivemeyer et al., 2011), productivity, and animal behavior (Breuer et al., 2000; Hemsworth et al., 2000). Manivannanan and Tripathi (2007) found that urban dairy farms have developed a more efficient dairy management through better and wider social contacts, better availability of inputs (veterinary services, concentrate feed), and a high commercial orientation (Lapar et al., 2010). Such economic motivation is the result of the union between the producer and the consumer. On one hand, the urban consumer prefers fresh, raw milk and relies on direct producer–consumer market, and on the other hand, the producer benefits from a more profitable sales channel. Moreover, the union between the producer and the consumer is strengthened by the sociocultural services provided by the cows to the Indian society, because as a sacred animal, it is still part of many religious ceremonies (Kennedy et al., 2018; Reichenbach et al., 2020). In contrast, rural farmers have based their management on lower commercial orientation, traditionalism, and scarcity of resources thus affecting their efficiency (Manivannanan and Tripathi, 2007). The combination of social and environmental components, within a so-called social-ecological system, has recently been introduced for livestock farming systems' classification (Martin-Collado et al., 2014). Rising megacities represent “hot spots” of complex and dynamic social-ecological systems because they are more vulnerable to environmental and anthropogenic hazards, and their socio-economic components are more diverse than those of smaller urban areas (Kraas, 2007). The social-ecological heterogeneity along rural–urban gradients in rising megacities might influence dairy production, a vital livestock sector in many regions of Asia and Africa (Prasad et al., 2019; Roessler et al., 2019).

World milk production will substantially increase in the next decade due to population and income growth (OECD-FAO, 2018). India especially will contribute to this development by 2027, with a global milk market share of 25% (OECD-FAO, 2018). In consequence, the Indian cattle population is continuously increasing, currently comprising more than 61 million dairy cattle (National Dairy Development Board, 2015). Productivity of dairy cattle is however low, ranging for example from 1.5 to 7 L per cow and day for Indian local Zebu cattle (Nivsarkar et al., 2000). To improve milk yields, exotic breeds, mainly Jersey and HF, have been imported during the past decades and used in pure breeding or crossbreeding schemes with local Zebu. In contrast to local Zebu cattle, high yielding Jersey and HF populations have mainly been selected for European and North American indoor production systems and are less adapted to harsh environments, while Zebu cattle have improved body temperature regulation abilities in response to heat stress (Hansen, 2004). Likewise, Al-Kanaan (2016) identified stronger environmental sensitivity (i.e. obvious productivity changes with environmental alterations) for HF compared to local dual-purpose populations in Germany. In terms of environmental sensitivity, trait responses have been studied in dependency of specific descriptors such as climate (Bouraoui et al., 2002), feeding strategies (Friggens et al., 2004), and husbandry systems (Young et al., 2014), without modelling descriptor combinations or interactions.

The aim of the present study was to extend the concept of environmental sensitivity through additional consideration of social descriptors. Against this background, we focused on novel functional trait recording along rural–urban gradients, choosing the rising megacity of Bangalore with its challenging environmental conditions and social complexity.

2. Materials and Methods

2.1. Study area

The study was carried out in the megacity of Bangalore, where population size doubled during the past 15 years, and 16 million inhabitants are expected in 2021 (Groupe SCE India, 2016). Bangalore is located in the southern Indian state of Karnataka at 12.97° northern latitude and 77.59° eastern longitude, 920 m above sea level. The climate is of tropical savannah type with two main periods: dry and humid. The humid monsoon period included June, July, August, and September. According to records from the Indian Meteorological Department (2015), the wettest month is September with an average total rainfall of 212.8 mm. The driest month is January with an average of only 1.9 mm rainfall. Two seasons can be distinguished in the dry period: a summer season including March, April, and May (daily temperatures between 20 and

34 °C), and a winter season including December, January and February (daily temperatures between to 16 and 31 °C; Indian Meteorological Department, 2015). As October and November are in-between the humid and dry period, both months were considered as autumn season.

2.2. Farm selection and description

To capture a variety of social-ecological settings, farms were randomly sampled from 31 different villages located along a northern and southern transect cutting through the city, following the survey stratification index (SSI) developed by Hoffmann et al. (2017) to characterize the rural–urban interface of Bangalore (Figure 3.1). The SSI considers build-up density (houses and infrastructures) and the distance to the city center and suggests the existence of three strata: “urban” (SSI < 0.3), “rural” (SSI > 0.5), and “(peri-)urban” or “mixed” (SSI: 0.3–0.5).

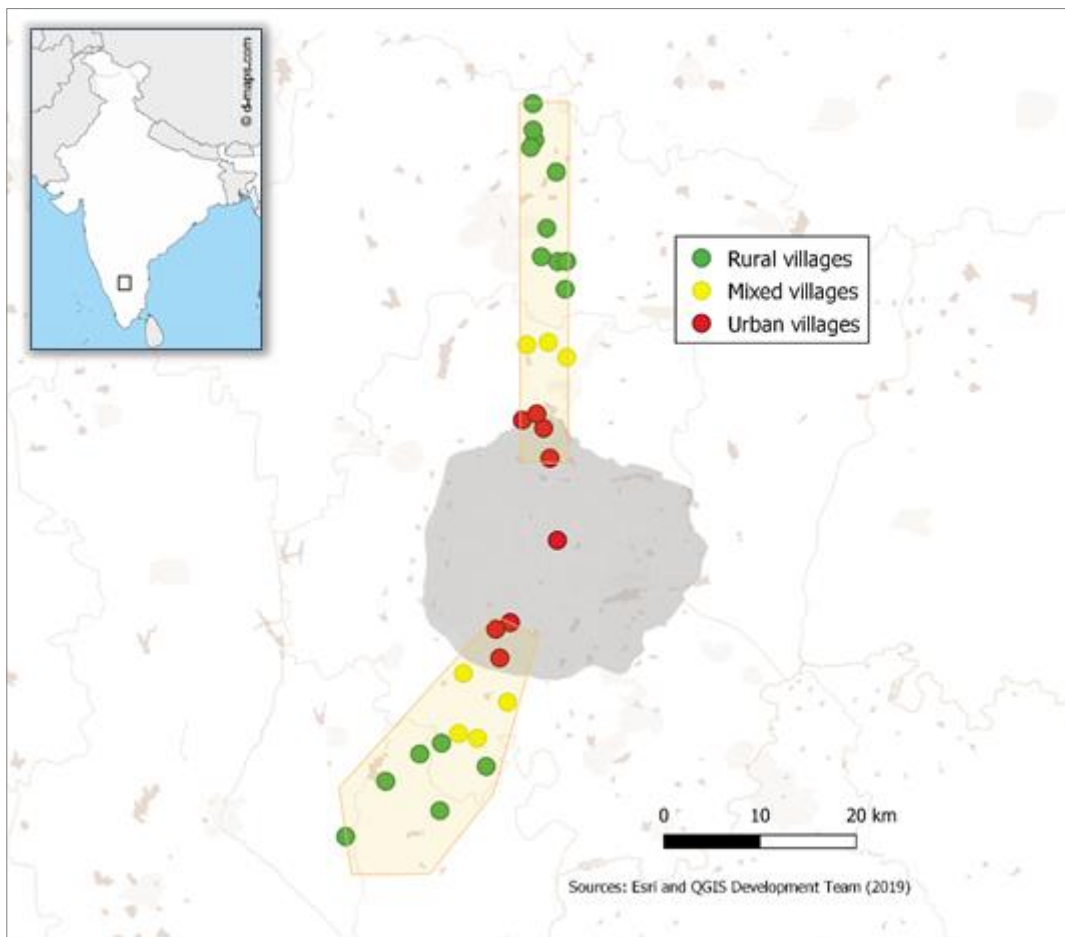


Figure 3.1. Map of Bangalore depicting the 31 sampled villages. The dark grey area represents the urban city zone. The orange contours indicate the northern and southern transects.

A total of 517 dairy cows, from 121 dairy farms in Bangalore, were considered in the present study. A two-step approach was applied to select the 121 dairy farms. In a first step, villages were selected semi-randomly, considering predefined percentages per SSI. In a second step, 20 to 30% dairy cow herds were randomly selected per village (300 herds), based on the latest vaccination list for foot-and-mouth disease. Only dairy cow herds with two or more dairy cows were sampled. The original 300 dairy cow herds were reduced to 121 herds after clustering into 4 groups based on coordinates for herd location, feeding strategies, and predominant herd genotypes. Aiming for a longitudinal trait data structure, each farm was visited three times between June 2017 and April 2018 in intervals of four months. Hence, the same cow was monitored at different lactation stages. Breeds present on the farms included HF, Jersey, native Zebu, “All Black” (synthetics breed from the cross between exotic (HF) sires with native cows), and a mixture of crossbreeds. All breeds were present in the three strata: urban, mixed, and rural. In the urban district, the number of cows per breed ranged from 5 for native and native crossbred to 40 for HF. The number of Jersey crossbred was lowest in the mixed district (7 cows); however, HF counted 80 cows. Moreover, HF was the dominant breed in the rural district (125 cows), and only seven native cows were observed in the same area. Herd size, at the time of selection, ranged from 2 to 12 dairy cows. The mean and range for herd size for the urban, the mixed, and rural district were 5.7 (2 to 12), 3.9 (2 to 8), and 3.7 (2 to 8), respectively. The average lactation number was 2.28. Daily temperature and humidity were recorded punctually on-farm at the time of visit with a weather station (HAMA 87682 LCD THERMO-/HYGROM. TH-200). The average temperature was 26.83 °C, ranging between 15.2 and 36.6 °C. Humidity ranged from 20% to 88%, with an average of 51.21%.

2.3. Cattle trait recording

Trait recording included daily milk yield (MY, in liters), body condition score (BCS), hygiene score of the udder (UddHS) and of upper legs (ULHS), hock assessment score (HAS), locomotion score (LS), subclinical mastitis (SubMast), and rectal temperature (RT, in °C). Body condition scores were assigned according to Ferguson et al. (1994) ranging from 1 (thin) to 5 (fat) with an increment of 0.25. According to Schreiner and Ruegg (2002), UddHS and ULHS ranged between 1 (completely clean) and 4 (completely covered with manure). Following Lombard et al. (2010), HAS score 1 was recorded for hocks with hair and without swelling, score 2 for hocks with hair loss and without swelling, and score 3 for hocks with hair loss and swelling. LS ranged from 1 to 5, with a higher score indicating poorer mobility and clinical lameness from 3 onward (Winckler and Willen, 2001). Subclinical mastitis (SubMast)

was detected utilizing the California Mastitis Test and followed the recording protocol of Kandeel et al. (2018). Hence, SubMast as a somatic count indicator was a binary trait, with a score = 0 for healthy cows and a score = 1 for abnormal milk reactions (sick cows). Descriptive trait statistics are summarized in Table 3.1. One trained person was responsible for all trait recordings.

Table 3.1. Descriptive statistics of daily milk yield, body condition score, udder hygiene score, upper legs hygiene score, hock assessment score, locomotion score, subclinical mastitis, and rectal temperature.

Trait	Variable Type	# Farm	# Cow	# Obs	Mean	SD	Min.	Max.
Milk yield (liter/day)	Numerical	121	469	945	10.67	5.46	1	35
Body condition score	Scale (1 to 5)	121	517	1138	2.75	0.37	2.00	4.00
Udder hygiene score	Scale (1 to 4)	120	478	936	2.03	1.04	1.00	4.00
Upper legs hygiene score	Scale (1 to 4)	121	484	942	2.61	1.11	1.00	4.00
Hock assessment score	Scale (1 to 3)	121	484	942	1.55	0.56	1.00	3.00
Locomotion score	Scale (1 to 5)	117	455	863	1.74	0.90	1.00	5.00
Subclinical mastitis	Binary	119	465	870	0.55	0.50	0	1
Rectal temperature (°C)	Numerical	121	463	874	38.51	0.65	36.2	41.3

(# = number of; Obs = Observations; SD = standard deviation; Min = minimum value; Max = maximum value).

2.4. Statistical models

A variety of linear mixed models, as implemented using the ‘lmer’ function in the lme4 package in R (Bates et al., 2015), were applied to analyze trait pattern in dependency of the continuous variable SSI. The model variety addressed investigations on the continuous variable SSI, which was studied via linear to quartic regressions. The general model 1, as defined for MY and BCS, is defined as follows:

$$y_{ijklmnop} = L_i + DIM_j + B_k + YS_l + \sum_{m=0}^q \alpha_m(SS_i) + F_n + cow_o + e_{ijklmnop} \quad (1)$$

Where $y_{ijklmnop}$ = MY or BCS; L_i = fixed effect for lactation number (1, 2, 3, 4, ≥ 5); DIM_j = fixed effect for days in milk classes (<3 months, 3–7 months, 7–12 months, > 12 months); B_k = fixed effect for breeds (native Zebu, HF, Jersey, All Black and their crossbreeds); YS_l = fixed effect for year-season (2017-Monsoon, 2017-Autumn, 2017-Winter, 2018-Winter, 2018-Summer); α_m = fixed regression coefficients (first to fourth orders Legendre polynomials (LP) in consecutive runs) for SSI, ranking from 0 to 1; F_s = random farm effect; cow_t = random cow

effect for repeated measurements; and $e_{ijklmnop}$ = random residual effect. Residuals for MY from model 1 were defined as adjusted MY for ongoing trait modeling strategies. Three milk yield classes (MYC) were defined based on adjusted milk yield: low, medium and high MYC, with a respective average (unadjusted) milk yield per class of 6.77, 9.96, and 15.52 L per cow and day.

Model 2 was designed for the health and wellbeing indicator traits, additionally considering interactions between productivity (MYC) and SSI. Temperature and humidity were only considered for the analysis of RT:

$$y_{ijklmnpstu} = L_i + DIM_j + B_k + YS_l + H_m + T_n + \sum_{p=0}^q \alpha_{rp} MYC_r(SSl) + F_s + cow_t + e_{ijklmnpstu} \quad (2)$$

where $y_{ijklmnpstu}$ = UddHS, ULHS, HAS, LS, SubMast or RT; H_m = fixed effect for environmental humidity classes (“low” = 0 to 30%, “medium” = 31 to 60%, and “high” = 61 to 90%); T_n = fixed effect for environmental temperature classes (“comfortable” $\leq 24^\circ\text{C}$, “stress” = 24 to 28 $^\circ\text{C}$, and “high stress” $\geq 28^\circ\text{C}$); MYC_r = fixed effect for adjusted MY classes (high, medium and low); and α_{rp} = fixed regression coefficients (first to fourth orders LP in consecutive runs) for MYC nested within SSI.

The Bayesian information criterion (BIC) derived from maximum likelihoods was used for model evaluations. Variance components and estimates for fixed effects from model 1 and 2 were based on restricted maximum likelihood.

3. Results

3.1. SSI modeling evaluations and general SSI impact

Models 1 and 2 showed the smallest BIC (indicating model superiority) when modeling continuous SSI with LP1 and LP4 (Table 3.2). The quartic function (LP4) was associated with the largest BIC values for all traits. Hence, regarding the following interpretations of trait pattern, we focus on results from LP1 and LP4 modeling. LP1 modeling for SSI was significant for MY, BCS, UddHS, ULHS, HAS, and RT ($p < 0.05$). None of the modeling strategies for SSI identified significant associations with the traits LS and SubMast. The breed effect significantly ($p < 0.05$) influenced MY, BCS, UddHS, ULHS, and HAS ($p < 0.05$; Table 3.3). However, the sampled number of native cattle was quite small with only 22 native cows in total (5 cows from the urban, 10 cows from the mixed, and 7 cows from the rural area).

Table 3.2. Model comparison from first to fourth orders Legendre polynomials (linear, quadratic, cubic and quartic) for milk yield (MY), body condition score (BCS), hygiene score of udders (UddHS) and of upper legs (ULHS), hock assessment score (HAS), locomotion score (LS), subclinical mastitis (SubMast) and rectal temperature (RT, in °C) via Bayesian information criterion (BIC).

Trait	BIC			
	Linear	Quadratic	Cubic	Quartic
MY	5473.45	5480.23	5484.85	5491.40
BCS	528.69	535.13	539.96	546.60
UddHS	2564.07	2582.42	2581.88	2601.95
ULHS	2564.31	2576.74	2581.22	2599.97
HAS	1473.67	1489.67	1504.57	1523.68
LS	1877.99	1888.09	1897.80	1915.46
SubMast	1315.60	1333.02	1351.69	1365.38
RT	1568.27	1621.32	1636.46	1651.11

Table 3.3. Least square means (LSMeans) and standard errors (SE) for milk yield (MY, in liters per cow and day), body condition score (BCS), hygiene score of udders (UddHS) and of upper legs (ULHS), hock assessment score (HAS), locomotion score (LS), subclinical mastitis (SubMast), and rectal temperature (RT, in °C) for different genotypes.

Breed	Traits	Traits							
		MY	BCS	UddHS	ULHS	HAS	LS	SubMast	RT
All Black	LSMeans	9.60 ^{ab}	2.69 ^{ab}	1.68 ^{ab}	2.39 ^{ab}	1.75 ^a	1.67 ^{ab}	0.56	38.39
	SE	0.55	0.04	0.12	0.12	0.06	0.12	0.06	0.07
All Black crossbreed	LSMeans	10.19 ^{ab}	2.67 ^{ab}	1.99 ^{ab}	2.46 ^{ab}	1.46 ^{bc}	1.49 ^a	0.64	38.49
	SE	0.63	0.05	0.13	0.13	0.07	0.14	0.07	0.08
Holstein Friesian (HF)	LSMeans	11.14 ^a	2.8 ^a	1.99 ^a	2.58 ^a	1.58 ^{ac}	2.01 ^a	0.61	38.45
	SE	0.38	0.02	0.08	0.09	0.04	0.07	0.03	0.05
HF crossbreed	LSMeans	9.51 ^{ab}	2.78 ^a	1.86 ^{ab}	2.46 ^a	1.76 ^a	1.92 ^a	0.61	38.47
	SE	0.56	0.04	0.12	0.12	0.07	0.12	0.06	0.07
Jersey	LSMeans	8.42 ^b	2.59 ^b	2.02 ^a	2.55 ^a	1.22 ^{bd}	1.76 ^{ab}	0.70	38.39
	SE	0.57	0.04	0.12	0.12	0.07	0.13	0.06	0.07
Jersey crossbreed	LSMeans	8.48 ^{bc}	2.67 ^{ab}	1.87 ^{ab}	2.41 ^{ab}	1.45 ^{abd}	1.56 ^{ab}	0.62	38.38
	SE	0.74	0.06	0.15	0.15	0.09	0.17	0.09	0.09
Native	LSMeans	5.16 ^c	3.11 ^c	1.35 ^b	1.85 ^b	1.28 ^{bc}	1.22 ^b	0.57	38.36
	SE	0.91	0.07	0.19	0.19	0.11	0.20	0.11	0.13
Native crossbreed	LSMeans	8.04 ^{b c}	2.83 ^{ac}	1.85 ^{ab}	2.36 ^{ab}	1.71 ^{ace}	1.44 ^{ab}	0.59	38.45
	SE	0.88	0.07	0.18	0.18	0.10	0.19	0.10	0.11

Different superscripts within the same column indicate significant genotype differences (P -value < 0.05).

3.2. SSI impact on milk yield and body condition score

Least square means for MY decreased along the SSI for both modeling strategies LP1 and LP4 (Figure 3.2, results from model 1). According to the linear SSI modeling, cows in urban areas produced an average of 10.04 L of milk per day, but cows in rural areas produced only 7.69 L per day. Likewise, the quartic SSI function (i.e. LP4) indicated a significant decline in MY for cows kept in rural areas. Within the urban areas, MY was at its lowest (8.40 L per day) for SSI 0.26. However, MY was lowest (7.44 L per day) across the quartic curve pattern in the very remote rural areas (SSI = 0.97). Variance components for MY and BCS using LP1 were 2.95 and 0.06 for the cow effect, 6.89 and 0.01 for the farm effect, and 11.44 and 0.04 for the residual effect, respectively. When changing the modeling strategies to LP4, the variance components were very similar.

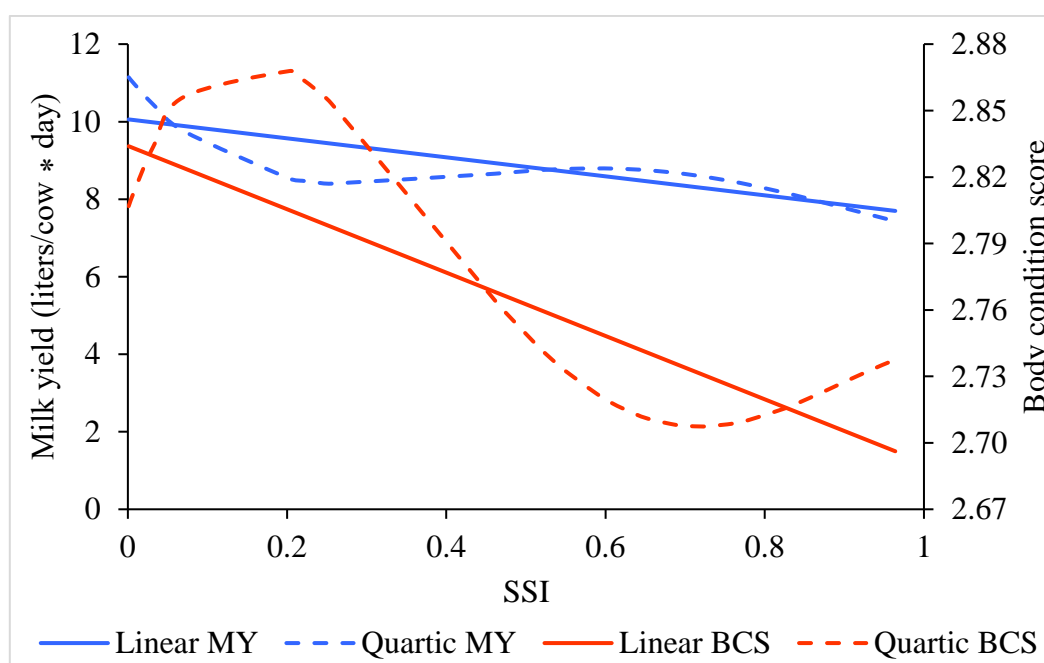


Figure 3.2. Least square means for milk yield (MY) and body condition score (BCS) in dependency of the survey stratification index (SSI).

As inferred for MY, the linear regression line (LP1) in model 1 for BCS decreased (Figure 3.2) with increasing SSI (from 2.83 at SSI 0 to 2.69 at SSI 1). However, the quartic curve pattern (LP4) showed a partly different trait response for MY and BCS in dependency of SSI. One example for the opposite BCS and MY trait responses is the highest BCS (2.87) at 0.2 and the lowest BCS (2.71) at SSI 0.7, while daily MY was similar at both SSI levels (8.53 L at SSI 0.2 and 8.59 L at SSI 0.7).

3.3. Impact of SSI on health and hygienic Score

Both hygiene scores UddHS and ULHS showed similar trait responses to SSI, with increasing SSI, UddHS, and ULHS, indicating that cattle were cleaner in urban than in rural areas (Figure 3.3, results from model 2). Linear regression lines for UddHS and ULHS in high and medium MYC were parallel, with the scores for UddHS smaller than ULHS. Variances explained by the cow, farm, and residual effects were 0.06, 0.36, and 0.57 for UddHS, respectively, and 0.00, 0.53, and 0.58 for ULHS, respectively.

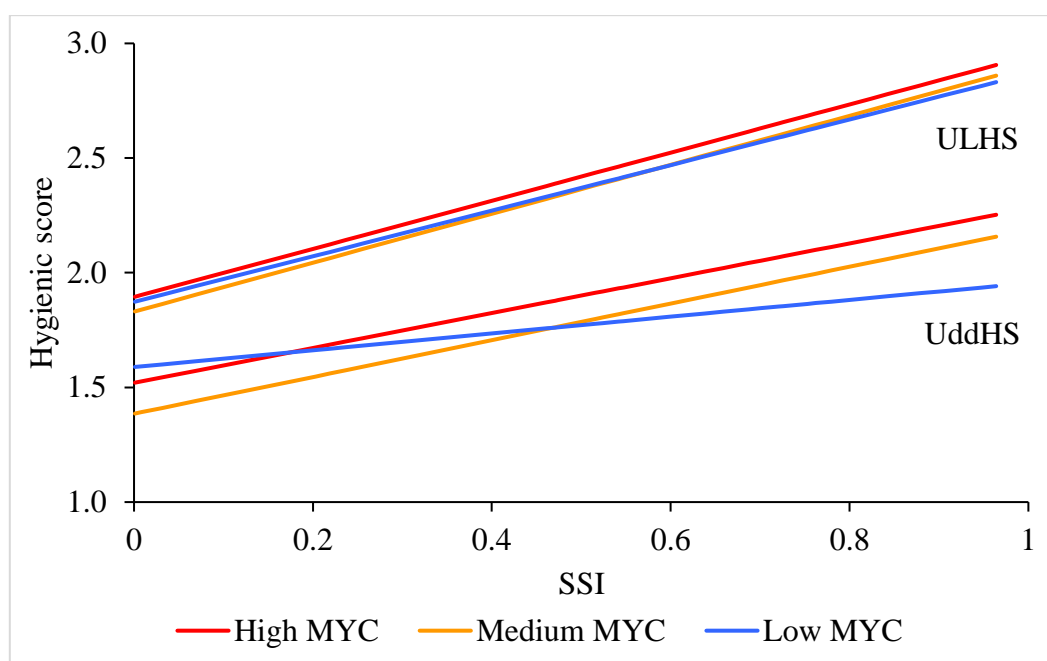


Figure 3.3. Least square means for upper leg hygiene score (ULHS) and udder hygiene score (UddHS) in dependency of the survey stratification index (SSI) and milk yield classes (MYC).

According to the linear modeling using model 2, HAS decreased gradually from urban to rural areas, indicating an improved leg health status for cattle kept in rural areas (Figure 3.4). Considering cow productivity, cows in the low MYC had a low prevalence for hock injuries across the SSI. HAS was the highest in urban district for cows in the high MYC, followed by cows in the medium MYC. As SSI increased, difference in HAS values between cows in the high and medium MYC decreased, reaching an equal HAS score in rural areas (SSI 0.82). Estimated variances for the cow, farm, and residual effects were 0.07, 0.04, and 0.16, respectively.

Model 2 was also used to analyze LS responses. With regard to the linear SSI modeling, LS for cows in the low MYC equaled 1.6 and was quite constant across the SSI (Figure 3.4). At SSI 0, LS was 1.83 for cows in the medium MYC and 1.80 for cows in the high MYC and decreased

to 1.53 and 1.46 at SSI 1. Interactions between MYC and SSI were not significant for LS. Variances were 0.45 for the cow effect, 0.14 for the farm effect, and 0.15 for the residual effect.

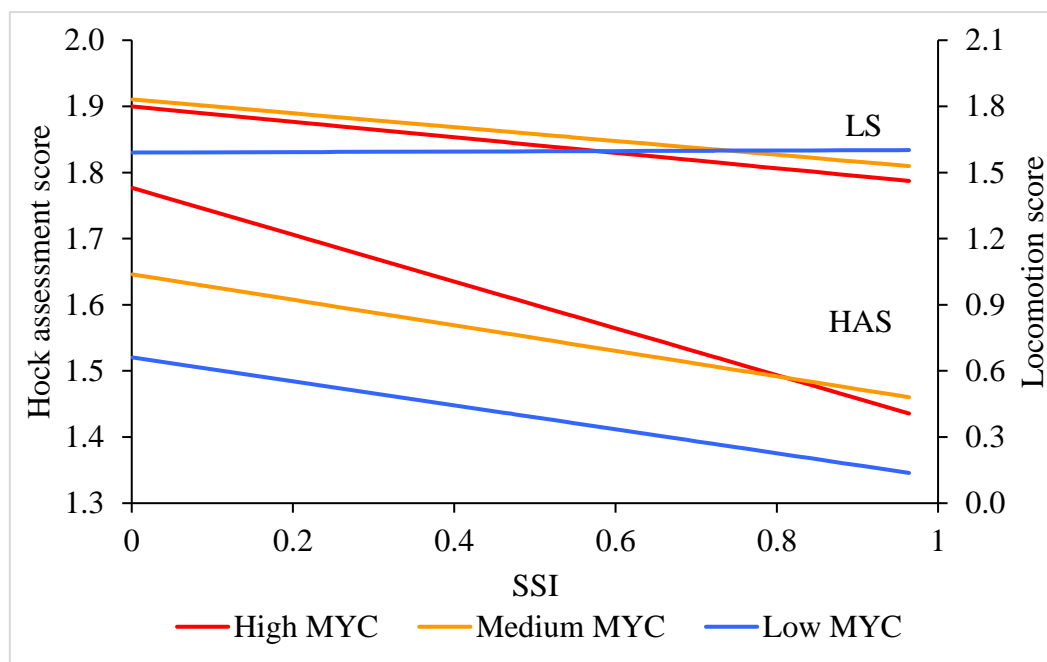


Figure 3.4. Least square means for hock assessment score (HAS) and locomotion score (LS) in dependency of the survey stratification system (SSI) and milk yield classes (MYC).

For SubMast, no significant interactions ($p > 0.05$) between MYC and SSI were identified. For cows in the high and medium MYC, the incidence of SubMast increased by 5% from SSI 0 to SSI 1 (Figure 3.5). Similar SubMast responses along the SSI (from 0 to 1) were observed for cows in the high and the medium MYC, i.e. an obvious increase of disease incidence from 0.59 to 0.64 and from 0.55 to 0.61, respectively. In urban areas, disease incidence was significantly higher for cows in the low MYC compared to cows in the high MYC (+16.99%). However, in rural areas, opposite effects were observed, i.e. lower disease incidences for the low MYC cow group (-4.86%). Variance components for SubMast were 0.08, 0.01, and 0.15 for the cow, farm, and residual effects, respectively.

3.4. Impact of SSI on rectal temperature

RT increased in dependency of SSI. This was the case for all MYC (Figure 3.6, results from model 2). In urban areas, least squares mean for RT of cows in the high MYC was highest (38.34 °C), followed by cows from the low (38.20 °C) and medium (38.14 °C) MYC. Between SSI 0.6 and SSI 0.8, trait response for RT was within the same range but diverged again slightly in rural areas ranging between 38.67 °C (medium MYC) and 38.57 °C (high MYC). Cow, farm, and residual variances for the RT were 0.00, 0.10, and 0.24, respectively.

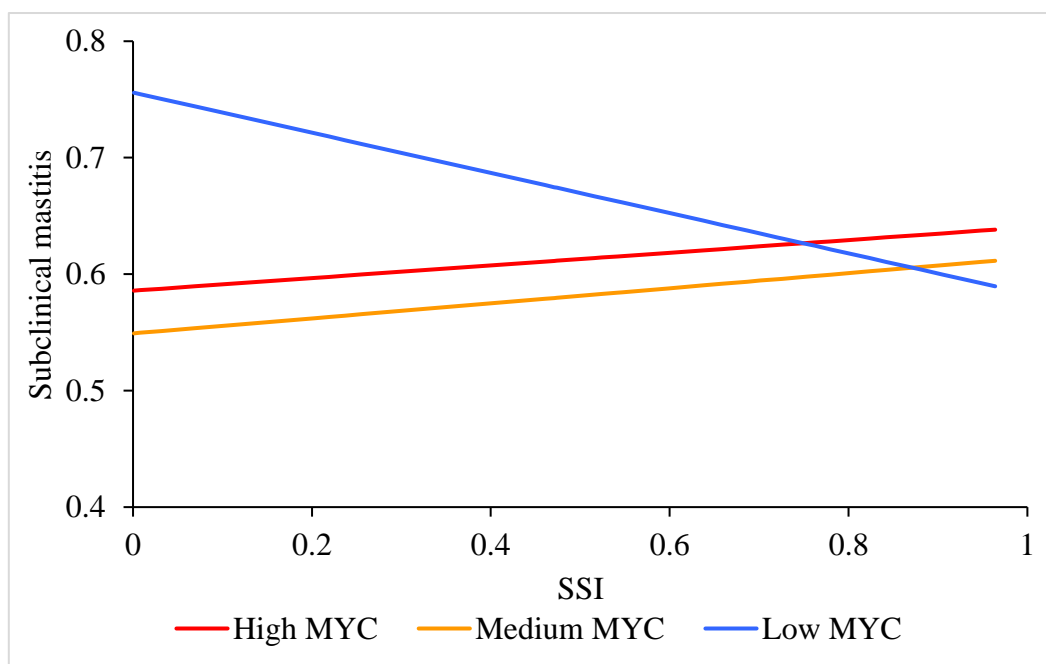


Figure 3.5. Least square means for subclinical mastitis in dependency of the survey stratification system (SSI) and milk yield classes (MYC).

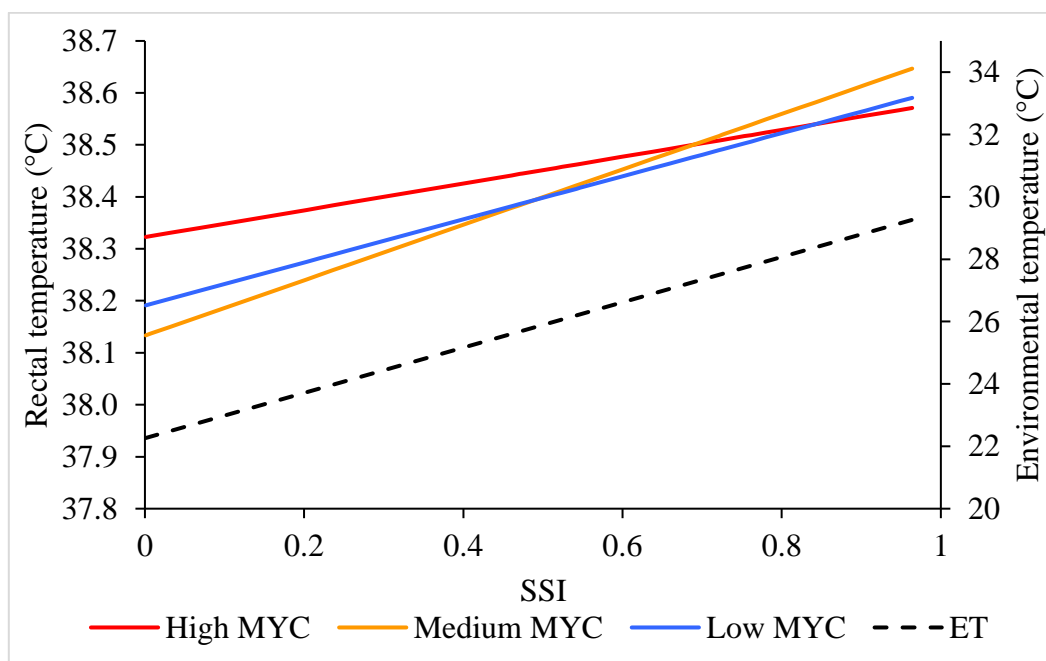


Figure 3.6. Least square means for rectal temperature and environmental temperature (ET) in dependency of the survey stratification system (SSI) and milk yield classes (MYC).

4. Discussion

4.1. Milk yield and body condition score in dependency of SSI

Across the whole SSI, MY was low with an average of 10.67 L per cow and day (Table 3.1), which is well below HF kept in European indoor production systems and reflects the harsh environment of Bangalore. However, the recorded MY is similar to the 7.1 kg of MY per day and crossbred cow kept in India for the 2011/14 period (Landes et al., 2017). A higher MY in

urban areas compared to rural or mixed ones is not a particularity for Bangalore, in the capital of Lesotho, the average MY per cow and day in urban farms was 12.77 L compared to 11.3 L in mixed farms (Gilles and Tawfik, 2001). However, in contrast to Bangalore, the price of milk was higher in mixed areas compared to urban ones (Gilles and Tawfik, 2001). Although urban and mixed dairy farmers face challenges like lack of space, fodder scarcity, labor shortage, and inappropriate disposal of animal excreta, they implement alternative management strategies to overcome such challenges. In this regard, they, e.g. focus on “free cattle feed” strategies, using organic kitchen waste from shops and neighbors with high nutritional value. These farmers have the freedom to set their own milk price, and the urban milk market is continuously increasing. Farmers in urban areas sell their milk directly to customers and decide their own price (0.35–0.45 €/L), whereas the milk collection centers in rural areas pay fixed milk prices in the narrow range from 0.29–0.31 €/L (own unpublished data). Despite the above-mentioned constraints, such social influences act as a stimulant for higher productivity in urban areas. Moreover, Indian urban dairy farms have developed an efficient dairy management through a commercial orientation and through consideration of veterinary services (Manivannanan and Tripathi, 2007). The commercial success of urban dairy cow farming is related to consumer preferences for fresh dairy products. Furthermore, milk quality is improved through consumer impact on the milk production chain (Lapar et al., 2010).

Despite the high MY in urban areas (Figure 3.2), exotic (49.2%) and crossbreed cows (47.3%) comprise a large percentage of milking cows. Jersey and HF with ancestors from Europe and North America are kept at the Livestock Breeding and Training Centre, Hesaraghatta, Bangalore (State Livestock Breeding & Training Centre, 2020), to produce frozen semen, since AI is the most common practice along Bangalore’s rural–urban gradient. Cattle pasturing without supervision in urban areas was common, implying an increasing risk of uncontrolled matings, which could explain the higher percentage of crossbreeds.

Parallel to MY, cattle kept in urban areas had an optimal BCS, supporting the notion that dairy farming is a rewarding business. Consequently, urban farmers put attention on health, nutrition, genetics, and management of their cows and have access to better inputs, with positive impacts on BCS, MY, UddHS, and ULHS. Moreover, compared to the rural areas, the share of illiterate herd owners in urban areas decreased by 50% between 1993 and 2009 (Agrawal, 2014). Kumbhakar et al. (1989) pointed out that well-educated farmers tend to enhance the productivity of their dairy cows. Partly opposite curve pattern for MY and BCS (quartic

regressions) in response to SSI might reflect physiological associations, i.e. lower BCS for high yielding cows due to the mobilization of body fat depots (Mushtaq et al., 2012).

4.2. Hygiene and health in dependency of SSI

Hygiene is of great importance for dairy farming, especially in urban areas, where the authorities want to restrict undesired by-products from dairy cattle farming, e.g. spread of diseases or environmental impact such as odor pollution and sewage (Nunan, 1999). Correspondingly, cows from dairy farms located in urban areas had superior hygiene scores, indicating that urban farmers were indeed paying more attention to hygiene, animal health, and wellbeing. Close spatial cohabitation of cows and humans was observed in the urban areas of Bangalore, which encourages farmers to improve hygiene and health management. In contrast, cows in the low MYC had poor scores for UddHS and ULHS at SSI 0, but were very clean at SSI 1. Atasever and Erdem (2009) reported similar hygiene scores and productivity of Holstein cows in response to social-ecological alterations in Turkey: both UddHS and ULHS were negatively correlated with daily MY.

The decrease of HAS and LS along the urban-to-rural gradient for all MYC might be related to stocking density, because increasing stocking density is associated with hock damages and lameness (Solano et al., 2015). In our study, the farms with higher herd size were located in urban areas, where land availability is scarce. Therefore, this could lead to a higher stocking density, which would explain that urban cows had poorer scores for HAS and LS. Moreover, the type of floor strongly influences hock health as well. In Bangalore, the floor of the sheds changed from mostly concrete floors in urban areas to sandy floors in rural areas. Singh et al. (1993) affirmed that prolonged standing time on concrete floors increases the predisposition to lameness. Norring et al. (2008) stated that the susceptibility to claw diseases and to hock lesions was lower on sand floors, compared to production systems with concrete floor and straw cover. Cows in high and medium MYC had higher incidences of hock lesions, supporting the antagonistic relationship between health and productivity (Juarez et al., 2003; Rutherford et al., 2008). In this regard, trait responses for HAS and LS were similar, confirming the strong and positive correlation between both traits (Sadiq et al., 2017). Mastitis is the most prevalent production disease in dairy herds (Seegers et al., 2003). Swami et al. (2017) reported an incidence of subclinical mastitis of 35.0% in 60 lactating cows from the Maharashtra state, India. Bangar et al. (2015) reviewed 25 articles on subclinical mastitis in dairy cows in India and reported a high variation for the incidence of subclinical mastitis between studies and geographical locations, in the range of 20.73 to 78.55%. In our study, the incidence of 55% fits

the review of Bangar et al. (2015), who estimated a prevalence of 46.35% of subclinical mastitis in 6344 dairy cows from 25 studies in 12 Indian states. Variations in subclinical mastitis prevalence are attributed to differences in herd size, animal management, farmer education, feeding strategy, agroclimatic conditions, and milk marketing (Joshi and Gokhale, 2006a). In the present study, the SubMast incidence slightly increased with increasing SSI for cows in the high and medium MYC, also reflecting the curve pattern for the two hygiene scores. Accordingly, Schreiner and Ruegg (2002) identified strong associations between hygiene scores and somatic cell counts for Holstein and Jersey populations located in Wisconsin, USA. For cows from the low MYC, the SubMast incidence was higher in urban than in rural areas. Strong detrimental impact of SubMast on cow production traits was reported in some previous studies (Bareille et al., 2003; Firat, 1993).

4.3. Rectal temperature in dependency of SSI

Narayan et al. (2007) measured an average RT of 38.6 °C for lactating HF x Sahiwal crossbred cows in an ambient temperature range between 23.2 and 34.2 °C in India. Burfeind et al. (2010) measured RT from 20 cows before and after defecation in Canada, and values were similar, i.e. 38.6 °C and 38.5 °C, respectively. In the present study, average RT was 38.5 °C, independent from the measurement date and herd location. Theoretically, RT increases with an increase of environmental temperature (McDowell, 1958). Along Bangalore's rural–urban gradients, ambient temperature in cow location increased with increasing SSI, because of the differences in shed type or the location where the cows rest. In urban areas, cattle are predominantly kept in the basement or ground floor of the house or in front of the house under a proper cement roof. In rural areas, only a simple wooden structure with some hay on top is used as shed, offering little protection against heat stress. In consequence, the increase of ambient shed temperature and RT in rural areas might be due to the scarcity of shadow and heat insulation (Sivakumar et al., 2017; Tucker et al., 2008). RT is also influenced by MY (Berman et al., 1985). Hence, an intensified metabolism at higher MY could explain the higher RT of cows in the high MYC in urban and mixed areas in comparison to lower RT of cows in medium and low MYC in the same areas.

4.4. Overall social-ecological impacts on dairy cattle Farming

Results from the present study showed an impact of SSI on a variety of important cow traits including productivity and functionality, such as MY, BCS, UddHS, ULHS, HAS, and RT. The SSI was constructed based on spatial information on buildings density and distance to the city

center (Hoffmann et al., 2017) and was thereby able to capture characteristics also relevant for dairy farming. While urban dairy production is common in India (Prasad et al., 2019), the breeding strategy of dairy farmers according to their location along rural–urban gradients is overlooked: in India, bulls are allowed to freely roam the city because of the social and cultural status of the cow and it is common for urban dairy farmers to let their cattle pasture in the city too, which increases the opportunities of uncontrolled mating. Thus, sociocultural norms influence the breeding of urban dairy cattle as captured by prevalence of exotic genotypes and crossbreed along the rural–urban gradients of Bangalore (Figure 3.7). Genotype interacts with further parameters of dairy production: on one hand, in urban areas, crossbreeds benefited from better management and health care, thus having a higher MY. Exotics genotypes, on the other hand, were more common in rural areas and had a lower MY despite better genetic potential, partly because of heat stress (Figure 3.7). Crossbreeds indeed benefited from more shadows in urban areas, but urban crossbreeding could be an advantageous strategy to tackle some of the harsher environmental conditions of urban areas. However, poorer HAS and LS scores in urban areas still need to be tackled, either through breeding or improvement of husbandry conditions.

	SSI strata		
	Urban	Mixed	Rural
Average herd size (number of cattle)	5.7	4.0	3.8
Exotic breeds in the herd (%)	49	61	68
Crossbreeds in the herd (%)	47	33	30
Milk production (liters per day)	11.3	10.6	10.6
Ambient temperature in shed (°C)	25	25	29
Daily exercise (% of farms)	72	67	36

Figure 3.7. Social-ecological aspects related to dairy farming along a survey stratification index (SSI).

The impact of the rural–urban gradients on traits of dairy cows shows the relevance of the underlying parameters of herd management and environmental conditions; their summary in an index such as the SSI allows for the creation of more complete and accurate socioecological models for the analysis of livestock production systems. If those models are established and the daily costs and revenues for a specific social-ecological level of livestock production are known, its economic efficiency can also be addressed in a location-specific manner (König et al., 2007). Authors should discuss the results and how they can be interpreted in perspective of

previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5. Conclusions

Differences in phenotypic trait expressions in dairy cows were identified along the rural–urban gradients of Bangalore. The additional consideration of SSI in trait analyses contributed to a deeper understanding of cow trait reactions on social-ecological challenges. Cows from rural farms showed an increased body temperature, which might indicate scarcity of shadow in these areas. In contrast, urban farms were characterized by the presence of dairy cows with higher MY, more optimal BCS, and better hygienic conditions, which reflects better management and an intensified market-oriented dairy production. However, cows on urban farms also showed poorer hock health, particularly those with higher MY. This suggests that dairy intensification in urban areas leads to higher productivity through better management but at the detriment of animal welfare.

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CHAPTER 4

Enteric Methane Emissions of Dairy Cattle Considering Breed Composition, Pasture Management, Housing Conditions and Feeding Characteristics along a Rural-Urban Gradient in a Rising Megacity

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1. Introduction

In the past 30 years, world milk production has increased by more than 52% from 542 million tons in 1990 to 828 million tons in 2017. In the same period, India has tripled its milk production from 54 to 176 million tons, becoming the world's largest milk producer with 21% of the global production (FAOSTAT., 2020). With more than 78 million dairy farms (International Farm Comparison Network, 2011) that are mostly smallholdings with 2 to 6 cows (International Farm Comparison Network, 2019), India hosts 18% of the global dairy cattle population (FAOSTAT., 2020). Moreover, urbanization in India has increased rapidly and the share of urban population doubled in the past 60 years (World Bank, 2018). Urban and (peri-)urban agriculture have contributed to food security and covered the demand for food products in these areas, e.g. with regard to milk consumption, by keeping dairy cattle close to urban areas (Prasad et al., 2019).

As a detrimental by-product, the livestock farming sector contributes 14.5% to the global greenhouse gas (GHG) emissions (Gerber et al., 2013), whereby enteric methane (CH_4) emitted by ruminant livestock is the largest source (17%) of global anthropogenic CH_4 emissions (Knapp et al., 2014). GHG emissions are expected to increase by 35% until 2050, especially in developing countries due to animal population growth driven by increased demands of meat and dairy products (Patra, 2014).

Several techniques have been developed to measure enteric CH_4 emissions from ruminants. Among them, the respiration chamber is considered the reference method although it is neither portable nor suitable for recording a large number of animals (Goopy et al., 2016). Under practical farming conditions, the Sulphur hexafluoride (SF_6) tracer technique (Grainger et al., 2007) has a high accuracy. Nevertheless, both CH_4 recording techniques are expensive and can interfere with animal behavior (Garnsworthy et al., 2019; Goopy et al., 2016). Sniffer methods like GreenFeed (C-Lock Inc., Rapid City, SD, USA) are portable and labor extensive, but require a certain extent of animal training (Garnsworthy et al., 2019). The laser methane detector (LMD) technique was first suggested by Chagunda et al. (2009b) for large-scale trait recording in the field. Since then, several studies applied the methodology in ruminants (Chagunda and Yan, 2011; Reintke et al., 2020; Ricci et al., 2014; Sorg et al., 2017). Even though concerns exist in view of repeatability and accuracy of the measurements (Garnsworthy et al., 2019; Pickering et al., 2015), due to its portability, low cost, ease of handling, non-invasiveness and non-interference with animal behavior, the LMD is currently the most flexible and simple method for on-farm determination of enteric CH_4 emissions.

The predictions of CH₄ emission from ruminant livestock traits is another option to calculate GHG emissions. Ricci et al. (2013) proposed a multiple equation approach including energy intake, physiological stage and the diet type to improve the precision of CH₄ predictions. Based on feeding characteristics and cow conformation traits, Yin et al (2015) predicted test-day methane emission via deterministic equations from cattle nutrition combined with stochastic simulations. Amount and quality of feed intake have a direct impact on enteric CH₄ emission (Hammond et al., 2009). The recorded CH₄ emission is also affected by the length of the time interval between cattle feeding and LMD recording (Chagunda, 2013; Ricci et al., 2014). Feed intake is associated with farm management practices and affected by environmental components. For example, season, ambient temperature and rainfall influence forage quality and possible access to pasture, and there is evidence that pasture-based dairy farming systems and confinement systems differ in enteric CH₄ output (O'Brien et al., 2014). Heat stress may be higher when animals graze on pasture than when staying in the barn and this affects feed intake (Rhoads et al., 2009) as well as animal health (Broaddus, 2001). Furthermore, Garnsworthy et al. (2012a) observed variations in CH₄ emissions depending on body weight (BW) and milk yield (MY) of dairy cows, whereby emissions were higher for cows with higher BW and MY. Although these effects are partly mediated by feed intake and feed composition, the higher metabolic rate of a heavier animal and/or an animal synthesizing more milk may also play a role (Yan et al., 2010). Thus, enteric CH₄ emission is the result of a variety of partly coupled physiological, environmental and managerial factors (Figure 4.1). In the urbanizing Indian context, an important element that integrates both environmental and social aspects is farm location within a rural-urban interface (Pinto et al., 2020). The spatial location of a farm does not only govern its physical access to resources such as pasture, but also plays a role concerning breed type kept, herd structure, housing space and housing structure (Pinto et al., 2020). These factors in turn shape the livestock management, and in further consequence they influence productivity as well as CH₄ emissions.

The combination of social and environmental components within a so-called social-ecological system has recently been proposed for livestock farming systems' classification (Martin-Collado et al., 2014). Rising megacities are hotspots of complex and dynamic social-ecological systems, because they are very vulnerable to environmental and anthropogenic hazards, and their social-economic components are more diverse than those of smaller urban areas (Kraas, 2007). In consequence, the aim of the present study was an assessment of the social-ecological responsiveness of enteric CH₄ emissions along a rural to urban gradient, choosing the Indian

megacity of Bangalore with its challenging environmental conditions, social complexity and large (peri-)urban dairy production (Prasad et al., 2019) as the research site.

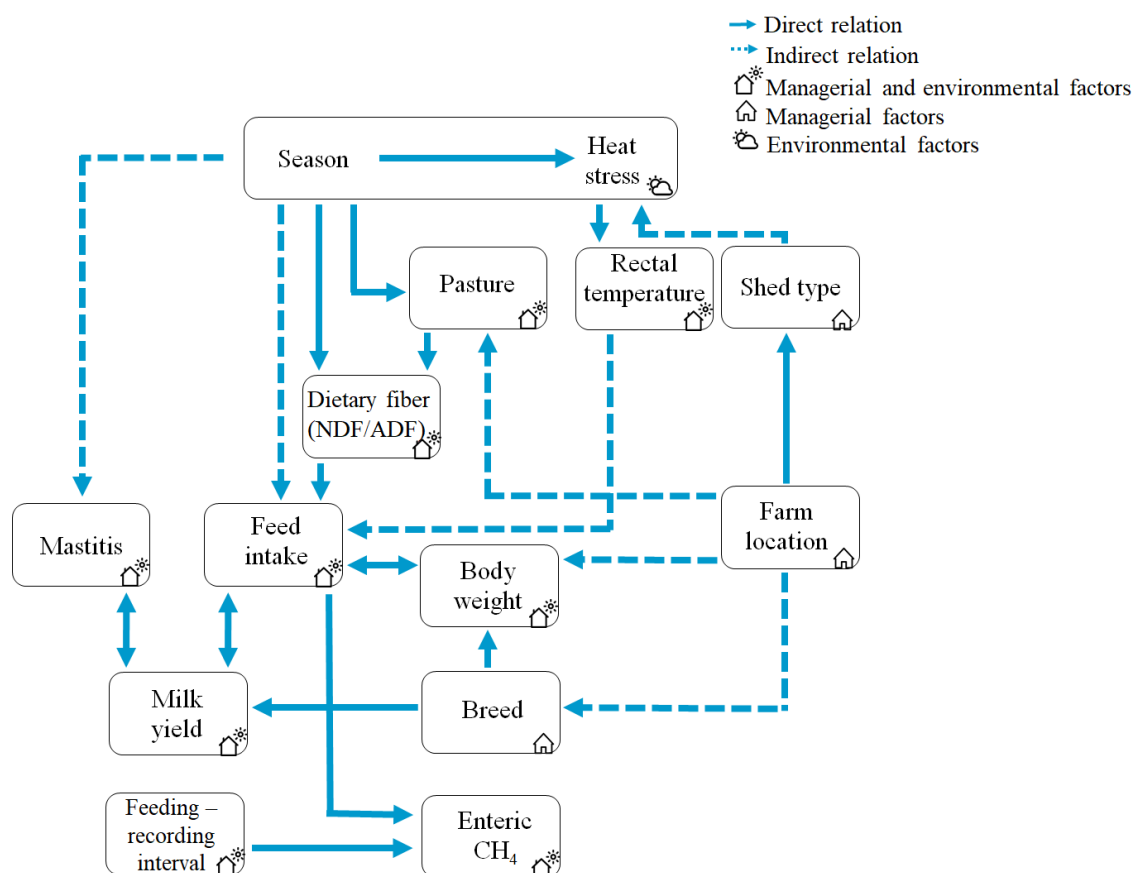


Figure 4.1. Conceptual framework depicting the relations of spatial, managerial and environmental factors with enteric methane emission across a rural-urban interface.

2. Materials and Methods

2.1. Farm selection and description

The study was conducted in Bangalore, southern India, where the population has doubled in the past 15 years and is expected to grow to over 12 million inhabitants by 2021 (Groupe SCE India, 2016). Dairy farms were sampled from 31 different villages located along a northern and southern transect in Bangalore (Figure 2). In order to study the influence of ecological gradients in combination with human-animal interaction, a survey stratification index (SSI) was developed by Hoffmann et al. (2017) for each geographical location within the mapped area. The SSI (between 0 and 1) considered the built-up density (houses and infrastructures) and the distance to the city center. In the current study, the SSI considered three SSI clusters: “urban” (SSI < 0.3), “rural” (SSI > 0.5), and “mixed” (SSI: 0.3 - 0.5). A two-step approach was applied to select the 119 dairy farms. In a first step, villages were selected semi-randomly, considering pre-defined percentages per SSI. In a second step, 20 to 30% dairy cow herds were randomly

selected per village (300 herds), based on the latest vaccination list for foot-and-mouth disease. To ensure continued, though minimal, participation in milk production, only dairy cow herds with two or more dairy cows were sampled. The original 300 dairy cow herds were reduced to 119 herds after clustered into four groups based on coordinates for herd location, feeding strategies and predominant herd genotypes (Pinto et al., 2020; Reichenbach, 2020). Each farm was visited three times between June 2017 and April 2018 in intervals of four months, resulting in 835 individual measurements at cow-level from 448 milking cows. The average herd size comprised 3 milking cows and ranged from 2 to 12 cows per farm.

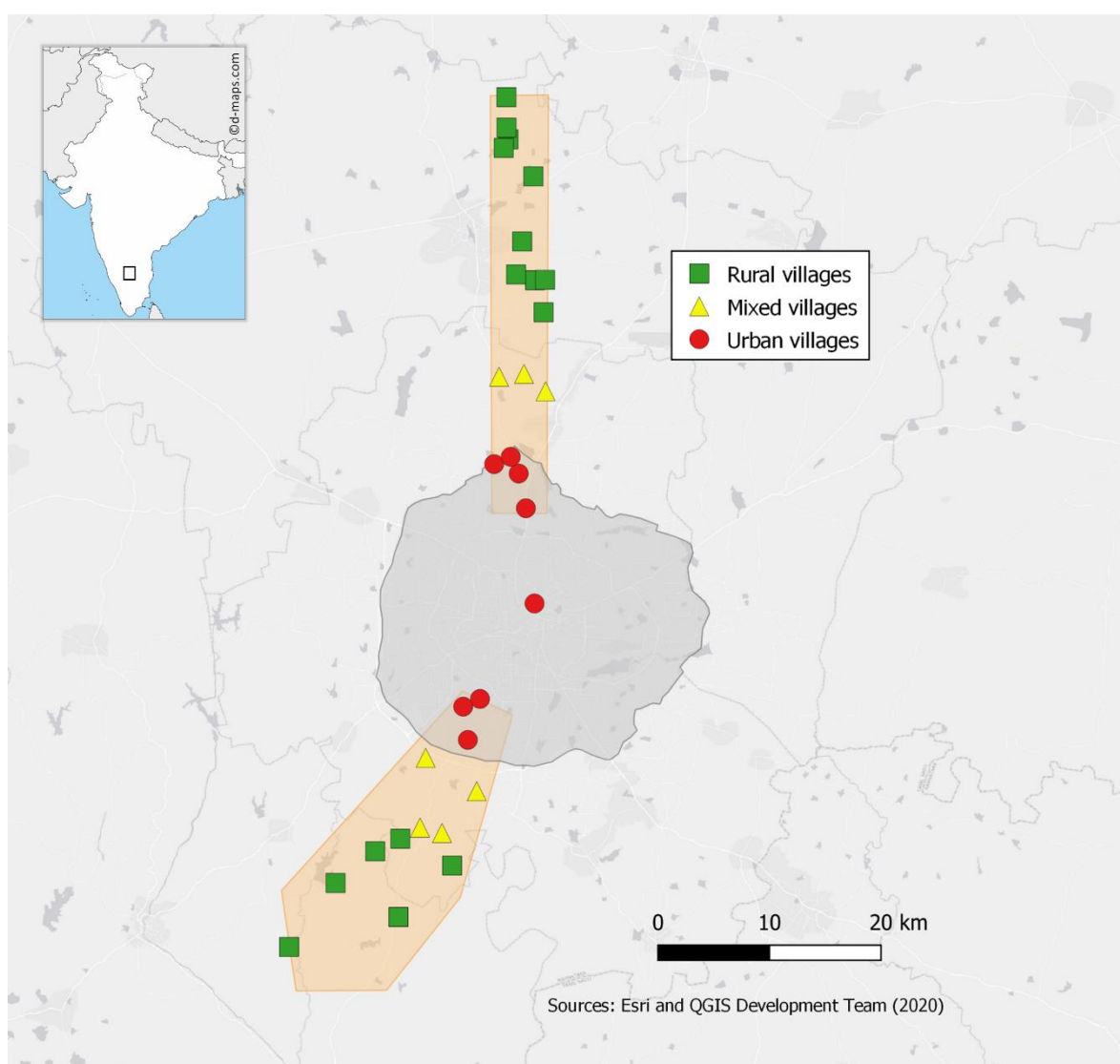


Figure 4.2. Map of Bangalore depicting the 31 sampled settlements. The dark grey area represents the urban city zone. The orange contours indicate the northern and southern transects.

Temperature and humidity were recorded at every farm visit using a portable weather station (HAMA 87682 LCD THERMO-/HYGROM. TH-200). The average temperature during the visits was 26.7°C, ranging from 15.2 to 35.9°C. Humidity on farms ranged from 20% to 88%, with an average of 54.7% across farms and visits. The temperature-humidity index (THI) per

farm and visit was calculated considering temperature (T) and relative humidity (RH) and applying the NRC (National Research Council (NRC)., 1971) formula:

$$THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH\%) \times (1.8 \times T - 26)$$

In Bangalore, access of cattle to pasture depended on farm location and varied with season, farmer's management strategy and health status of the cow. In this regard, at every visit, the pasture access (Past: yes, no) was recorded for each animal. Percentages of measurements with access to pasture in the three SSI clusters are listed in Table 4.1. None of the farms had ad libitum access to water, however, they offered water at milking time (15 L approx. twice a day) along with the concentrates.

2.2. Cattle production and conformation traits

Milk yield (MY), heart girth (HG) and body condition score (BCS) were recorded for all cows. BCS was subjectively scored on a linear scale ranging from 1 (thin) to 5 (fat) in increments of 0.25 (Ferguson et al., 1994). Out of 448 cows, 285 were exotic breeds (Holstein Friesian and Jersey), 13 were native breeds (Zebu cattle, mainly of the breed Hallikar) and 150 were first and multigenerational crossbreeds (exotic x native). Due to significant correlations between CH₄ and BW in previous studies (Garnsworthy et al., 2012a), BW of cows at recording was predicted based on the conformation traits HG, BCS, age, breed and pregnancy status. according to the following equation (Grund, 2018):

$$BW = \exp(-7.35492 + (2.55408 \times \ln(HG)) + (0.04043 \times \ln(\text{age})) + (\beta_1 \times BCS) + (\beta_2 \times \text{breed}) + (0.024741 \times \text{pregnant}) + 0.08317)$$

where HG = heart girth measured in cm; age = age at measurement (in month); β_1 = linear regression coefficient for BCS; BCS = body condition score; β_2 = regression coefficient for breed; breed = exotic, native or crossbreed; pregnant = 1 (pregnant) or 0 (non-pregnant).

The average lactation number was 2.9 and the maximum lactation number was 12. On average, daily MY, BCS, and BW from the 835 measurements were 10.67 liters, 2.65 points, and 383.29 kg, respectively. Average daily MY and BW in the three clusters are displayed in Table 4.1.

Table 4.1. Percentage of measurements per breed, pasture access (Past), measurement location (Loc), average milk yield (MY) and body weight (BW) for each survey stratification index (SSI) cluster.

SSI cluster	No. of measurements	Breed (%)			Past (%)			Loc (%)		MY (l/day)	BW (kg)
		Exotic	Crossbreed	Native	No	Yes	Indoor	Half-outdoor	Outdoor		
Urban	191	51.3	45.6	3.1	29.3	70.7	46.1	16.7	37.2	11.5	392.6
Mixed	250	62.0	31.2	6.8	34.4	65.6	32.0	11.2	56.8	10.5	382.8
Rural	394	68.3	31.5	0.2	62.7	37.3	22.6	29.7	47.7	10.4	379.1

2.3. Methane spot measurements

The CH₄ concentration in the exhaled air per cow was measured using the LMD, model Laser Methane mini (Tokyo Gas Engineering Solutions Corporation, 2015) considering windless environmental conditions, so that wind did not disturb the CH₄ measurements. The measurement location (Loc) was classified into outdoor, indoor and half-outdoor according to the type of shed, in which CH₄ emissions from individual cow were measured: the indoor class depicted a closed room with four walls and a roof, with or without ventilation (Figure 4.3a); The outdoor class was assigned when cattle were kept on an open field or in a shed without walls (Figure 4.3c); Half-outdoor was in-between the outdoor and the indoor classification, e.g. a shed with only two walls where air could freely circulate (Figure 4.3b). Percentage of measurements per measurement location for each SSI cluster is shown in Table 4.1.



Figure 4.3. Measurement location for methane emissions: (a) Indoor, (b) half outdoor and (c) outdoor location.

The LMD was programmed to record the CH₄ concentration in narrow intervals of 0.1 seconds. To capture the exhaled air, the green laser guide light from the LMD was pointed at the animal's nostrils for 2 minutes, implying single data set per measurement including 1200 CH₄ concentration values. Measurements were taken once per cow and visit, by milking time and at least 2 h after feeding. Thus, the mean time interval from cow feeding until the CH₄ recording was 3.85 h with a standard deviation of 3.56 h. The LMD automatically identified and recorded errors in the reflectance of the (invisible) laser beam occurring at any 0.1 seconds. These erroneous values were manually deleted from the data set upon processing. The recorded unit for CH₄ concentration was ppm x meter, but since the distance between the nostrils of the animal and LMD was exactly 1 meter, all values were expressed in ppm. A distance range laser was used to ensure the 1-meter distance between the nostrils and the LMD.

In a first step, all CH₄ measurements were corrected for the background environmental CH₄ at the measurement location. The minimum CH₄ concentration for each individual measurement and visit was set as environmental CH₄, which was subtracted from all other values of the

respective data set. Afterwards, the corrected measurements were categorized into mini-peaks and mini-troughs (Ricci et al., 2014). Only mini-peaks were kept for further calculations, and the remaining measurements were assumed to represent a mixture of two normal distributions with different means and variances, which correspond to two physiological paths of CH₄ emissions in ruminants, i.e. respiration and eructation of CH₄ (Blaxter and Joyce, 1963; Murray et al., 1976). Therefore, the CH₄ concentrations were separated into lower (the one with lower mean) and upper (the one with greater mean) normal distributions according to thresholds defined at 10% cumulative probabilities of the upper normal distribution for every individual and visit (Ricci et al., 2014). The thresholds for every individual measurement were estimated based on the “mixtool” package in R (Benaglia et al., 2009). Measurements lower than the thresholds were defined as respiration CH₄ and the remaining measurements as eructation CH₄. The average share of eructation CH₄ was 77.18% and for respiration CH₄ 22.82% in line with previous studies (Blaxter and Joyce, 1963; Murray et al., 1976). Means (absolute values) of overall CH₄ (AllMean, including respiration and eructation measures), respiration CH₄ (RespMean) and eructation CH₄ (ErucMean) were calculated by averaging the measurements per individual and visit according to the three definitions. Thereafter, the interval between starting and ending the LMD measurement was calculated and termed “measurement duration”. The sum of all mini-peaks for every individual and visit was further divided into the respective measurement duration to calculate overall CH₄ (AllMinute, including respiration and eructation measures), respiration CH₄ (RespMinute) and eructation CH₄ (ErucMinute). Finally, maximum respiration CH₄ (RespMax) and eructation CH₄ (ErucMax) were determined for each measurement. Descriptive statistics for the eight CH₄ traits are listed in Table 4.2.

2.4. Nutrition monitoring subsample

Nutritional monitoring was carried out in 27 out of the 119 selected herds, with 8 herd visits in intervals of 6 weeks during the study period (Reichenbach, 2020). The green fodder component included tall African maize (*Zea mays*) and Napier's hybrid grass (*Pennisetum glaucum* × *P. purpureum*). Urban dairy farmers additionally used organic wastes (vegetable and fruit peels) for feeding their cattle. Dry forages were mostly straw of finger millet (*Eleusine coracanal*) and dried maize. Concentrate feed contributed to 27-28% of the daily dry matter (DM) intake. On average, the cattle stayed on pasture for 5.9 ± 2.3 hours (Reichenbach, 2020). Feed type and quantitative feed intake of each individual cow in these herds were recorded. Fodder samples were analyzed for neutral detergent fiber (NDF) concentration according to Van Soest et al. (1991) at the National Institute of Animal Nutrition and Physiology (NIANP) in Bangalore.

Organic waste, pasture biomass, green and dry forages had an average fiber concentration of 482, 669, 675 and 708 g NDF kg⁻¹ DM, respectively (Reichenbach, 2020). Descriptive statistics for the eight CH₄ traits as determined in the nutrition subsample are listed in Table 4.3.

Table 4.2. Descriptive statistics of the defined methane emission traits

CH ₄ trait	# Farm	# Cow	# Measurement	Methane emission (ppm)			
				Mean	SD	Min.	Max.
AllMean	119	448	835	42.8	34.92	8.2	351.7
AllMinute	119	448	835	3969.0	2446.35	771.1	23785.2
RespMean	119	448	835	16.0	17.21	1.0	160.3
RespMax	119	448	835	48.4	81.40	1.0	900.0
RespMinute	119	448	835	879.9	1249.82	0.5	12873.9
ErucMean	119	448	835	108.1	148.28	9.7	1425.0
ErucMax	119	448	835	410.2	371.99	31.0	4152.0
ErucMinute	119	448	835	3089.0	2253.80	106.0	22098.0

= Number of; SD = Standard Deviation; Min = minimum value; Max = maximum value; AllMean = average all methane; Allminute = all methane per minute; RespMean = average respiration methane; RespMax = maximal respiration methane; RespMinute = respiration methane per minute; ErucMean = average eructation methane; ErucMax = maximal eructation methane; ErucMinute = eructation methane per minute.

Table 4.3. Descriptive statistics of methane emission from 27 farms with nutrition information.

CH ₄ trait	# Farm	# Cow	# Measurement	Methane emission (ppm)			
				Mean	SD	Min.	Max.
AllMean	27	78	146	37.2	25.00	8.2	119.2
AllMinute	27	78	146	3645.0	2110.67	905.2	11353.7
RespMean	27	78	146	13.3	12.86	1.0	74.8
RespMax	27	78	146	48.6	89.58	1.0	721.0
RespMinute	27	78	146	891.0	1207.36	0.5	7550.2
ErucMean	27	78	146	114.2	174.42	10.1	1271.2
ErucMax	27	78	146	489.1	537.96	39.0	4152.0
ErucMinute	27	78	146	2754.1	1993.99	106.0	10747.6

= Number of; SD = Standard Deviation; Min = minimum value; Max = maximum value; AllMean = average all methane; Allminute = all methane per minute; RespMean = average respiration methane; RespMax = maximal respiration methane; RespMinute = respiration methane per minute; ErucMean = average eructation methane; ErucMax = maximal eructation methane; ErucMinute = eructation methane per minute.

2.5. Statistical models

A linear mixed model implemented in R package “lme4” (Bates et al., 2015) was applied to analyze the CH₄ traits. The basic statistical model was:

$$y_{ijklmno} = \mu + \left(\frac{MY}{BW} \times 100\right)_i + B_j + THI_k + FasT_l + F_m + cow_n + e_{ijklmno} \quad (1)$$

where $y_{ijklmno}$ = logarithm transformation of the CH₄ traits; μ = overall mean effect; $\left(\frac{MY}{BW} \times 100\right)_i$ = fixed effect for MY/BW ratio in liter per kg multiplied by 100; B_j = fixed effect for breeds (exotic, crossbreed and native); THI_k = fixed effect for temperature-humidity index; $FasT_l$ = fixed effect for the time interval from cow feeding until the CH₄ recording (in hours); F_m = random farm effect; cow_n = random cow effect for repeated measurements; $e_{ijklmno}$ = random residual effect.

Following the model 1, three different variables, i.e. SSI cluster, pasture access and measurement location, were included one by one into the basic statistical model:

$$y_{ijklmnop} = \mu + \left(\frac{MY}{BW} \times 100\right)_i + B_j + THI_k + FasT_l + TestEff_m + F_n + cow_o + e_{ijklmnop} \quad (2)$$

where $TestEff_m$ = included the fixed effects SSI cluster (rural, mixed, urban), Past (yes, no) and Loc (outdoor, half-outdoor and indoor) in consecutive runs.

For the nutrition monitoring subsample, one additional variable was included as $TestEff_m$ in model 2, where $TestEff_m$ = level of NDF intake per metabolic weight ($MW = BW^{0.75}$; in g NDF/kg MW) on the day of visit. Two NDF intake levels were distinguished: high (NDF \geq 94 g/kg MW, $n = 71$ measurements) and low (NDF $<$ 94 g/kg MW, $n = 75$ measurements).

The R package “emmeans” (Lenth et al., 2018) was applied to estimate least square means (LSMeans) of levels within different effects.

3. Results

3.1. Basic environmental factors on CH₄ concentrations

From the basic statistical model 1, the MY/BW ratio significantly influenced AllMean (Table 4.4). Except for RespMinute and ErucMax, an increase in the MY/BW ratio was associated with an increased CH₄ concentration. The fixed effect THI significantly influenced the eructation CH₄, i.e. ErucMean, ErucMinute and ErucMax (Table 4.4). However, ErucMean and ErucMax decreased with THI and ErucMinute had a positive regression coefficient of 0.02.

With the prolongation of the time interval from feeding to CH₄ recording, AllMean, ErucMean and ErucMax decreased significantly. The fixed effect breed was not significant for the CH₄ traits. However, exotic breed had lowest values for all CH₄ traits, except for ErucMinute. Crossbreed showed highest LSM means for ErucMean and for the respiration CH₄, including RespMean, RespMax and RespMinute (Table 4.4).

Table 4.4. Regression coefficients of milk yield to body weight ratio (MY/BW), temperature humidity index (THI) and time interval from feeding to methane recording (FasT) and least square means of breed levels for methane emission (CH₄) traits.

CH ₄ trait	Fixed effects					
	MY/BW ¹	THI ¹	FasT ¹	Breed ²		
				Exotic	Crossbreed	Native
AllMean	0.03 [*]	-0.00 ^{ns}	-0.01 [*]	33.19 ^a	33.33 ^a	35.38 ^a
AllMinute	0.02 ^{ns}	-0.01 ^{ns}	-0.01 ^{ns}	3328.17 ^a	3351.99 ^a	3550.51 ^a
RespMean	0.03 ^{ns}	-0.01 ^{ns}	-0.00 ^{ns}	10.09 ^a	10.88 ^a	10.81 ^a
RespMax	0.03 ^{ns}	-0.00 ^{ns}	-0.02 ^{ns}	19.80 ^a	23.63 ^a	21.48 ^a
RespMinute	-0.01 ^{ns}	0.01 ^{ns}	0.00 ^{ns}	275.74 ^a	344.81 ^a	284.45 ^a
ErucMean	0.02 ^{ns}	-0.03 ^{**}	-0.03 ^{**}	61.50 ^a	70.33 ^a	70.31 ^a
ErucMax	-0.01 ^{ns}	-0.04 ^{***}	-0.04 ^{***}	296.92 ^a	305.91 ^a	358.25 ^a
ErucMinute	0.04 ^{ns}	0.02 [*]	-0.00 ^{ns}	2406.74 ^a	2242.53 ^a	2313.07 ^a

¹ = regression coefficient; ² = LSM means; ns = not significant; * = *P*-value < 0.05; ** = *P*-value < 0.01; *** = *P*-value < 0.001; Different letters in the same row indicate significant difference between breed levels (*P*-value < 0.05); AllMean = average all methane; Allminute = all methane per minute; RespMean = average respiration methane; RespMax = maximal respiration methane; RespMinute = respiration methane per minute; ErucMean = average eructation methane; ErucMax = maximal eructation methane; ErucMinute = eructation methane per minute.

3.2. Impact of measurement location on CH₄ concentrations

The location where the LMD measurement took place also had an effect on CH₄ concentrations. LSM means for AllMean, AllMinute, RespMean, RespMax, RespMinute, ErucMean and ErucMinute were significantly higher (*P*-value < 0.05) in the indoor class, followed by the half-outdoor and the outdoor classes (Figure 4.4). For ErucMax, the CH₄ concentration was 356.45 ppm in half-outdoor and decreased to 313.05 ppm in indoor and to 308.78 ppm in outdoor. No differences were detected between locations for ErucMax. Significant differences in CH₄ between indoor and outdoor classes were observed for AllMean (16.13 ppm), AllMinute

(870.26 ppm/minute), RespMean (7.70 ppm), RespMax (14.19 ppm), RespMinute (131.52 ppm/minute), ErucMean (25.47 ppm) and ErucMinute (587.39 ppm/minute).

3.3. Impact of access to pasture on CH₄ concentrations

Pasture access was an important factor, because LSMeans for AllMean, AllMinute, RespMean, RespMax, RespMinute, ErucMean and ErucMinute were significantly higher (P -value < 0.05) for cows having no access to pasture (Figure 4.5). The differences between CH₄ concentrations in cows with and without access to pasture were 6.01 ppm for AllMean, 541.26 ppm/minute for AllMinute, 3.26 ppm for RespMean, 7.96 ppm for RespMax, 132.71 ppm/minute for RespMinute, 19.00 ppm for ErucMean, and 55.63 ppm for ErucMax.

3.4. Impact of fibre intake on CH₄ concentrations

No significant impact on the CH₄ traits were identified with respect to the daily NDF intake level, even though LSMeans for all CH₄ traits were numerically higher at the high NDF intake level (Figure 4.6). The differences in CH₄ concentrations between cows with high and low NDF intake level were 3.93 ppm for AllMean, 425.03 ppm/minute for AllMinute, 1.40 ppm for RespMean, 4.64 ppm for RespMax, 49.84 ppm/minute for RespMinute, 16.64 ppm for ErucMean, 71.34 ppm for ErucMax, and 85.22 ppm/minute for ErucMinute.

3.5. Impact of SSI cluster on CH₄ concentrations

LSMeans for AllMean, AllMinute, RespMean, RespMax, RespMinute, ErucMean and ErucMinute were highest in the urban areas, followed by the rural and the mixed areas. For ErucMax, the highest CH₄ concentration was observed in the mixed areas (Figure 4.7). The fixed effect SSI cluster had a significant impact (P -value < 0.05) between urban and mixed areas on both AllMean and RespMean while between urban and rural areas impact was only significant on AllMean. The differences in CH₄ concentrations between urban and mixed areas were 10.31 ppm for AllMean and 3.89 ppm for RespMean.

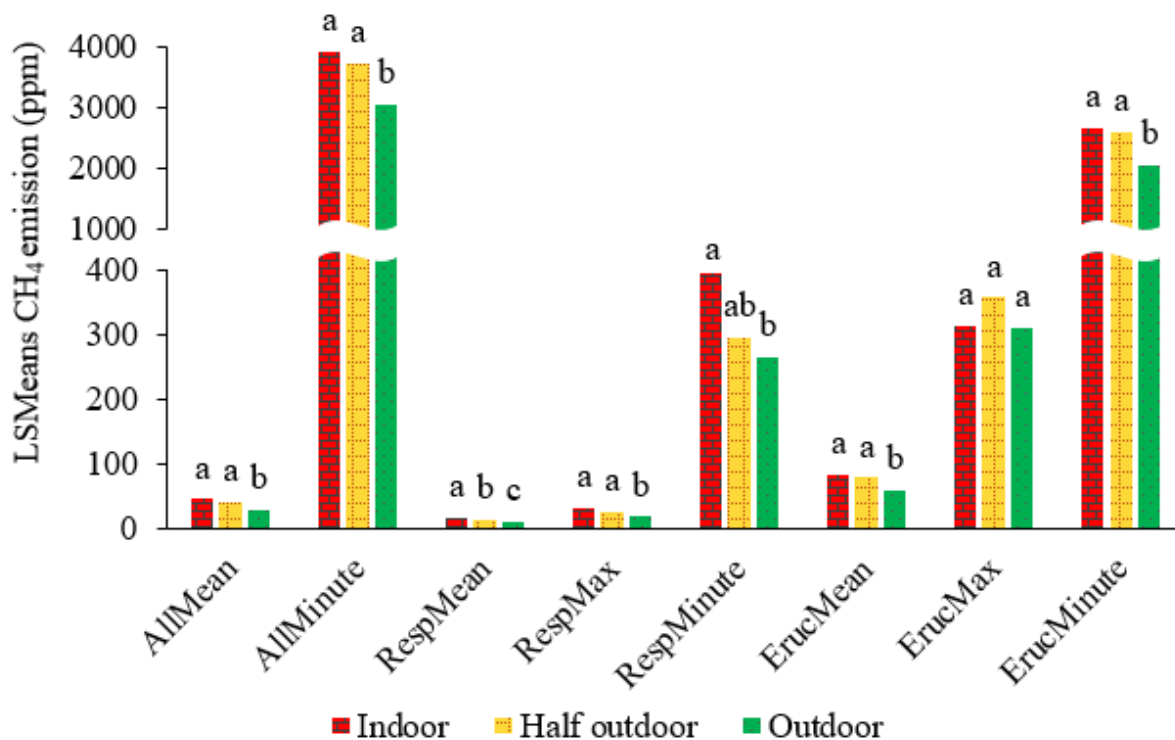


Figure 4.4. Least square means (LSMeans) for the methane traits in indoor, half-outdoor and outdoor classes. Different letters on bars indicate significant differences (P -value < 0.05).

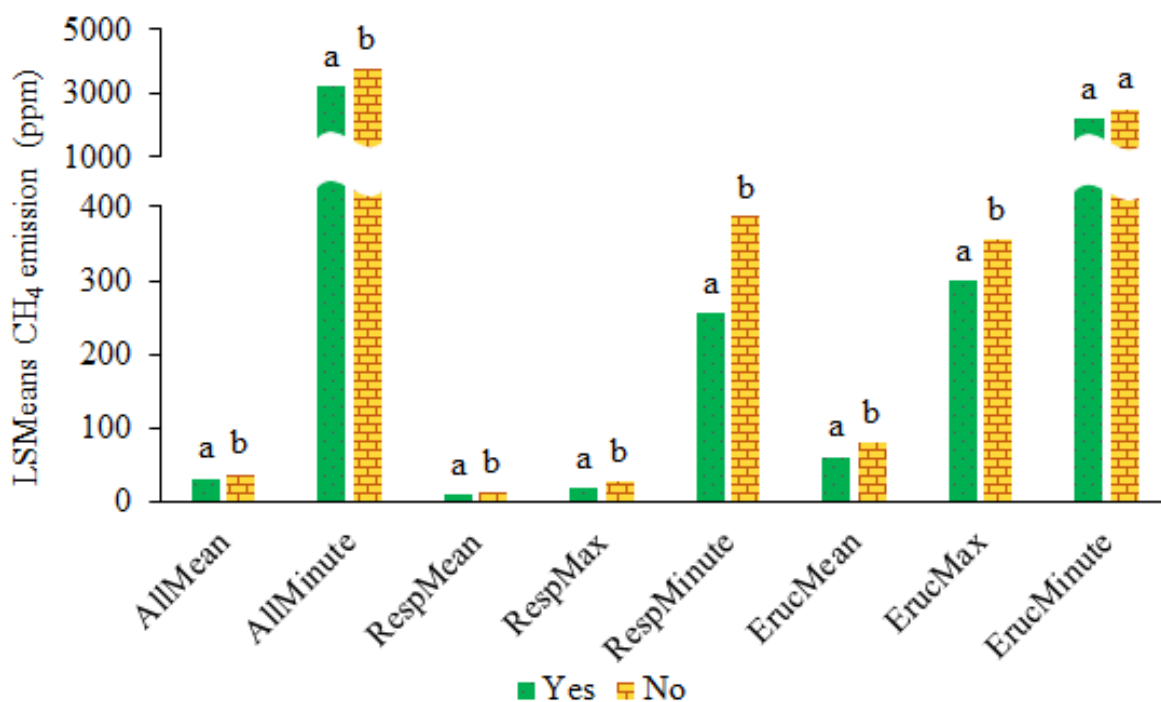


Figure 4.5. Least square means (LSMeans) for the methane traits in classes with (Yes) and without (No) access to pasture. Different letters on bars indicate significant differences (P -value < 0.05).

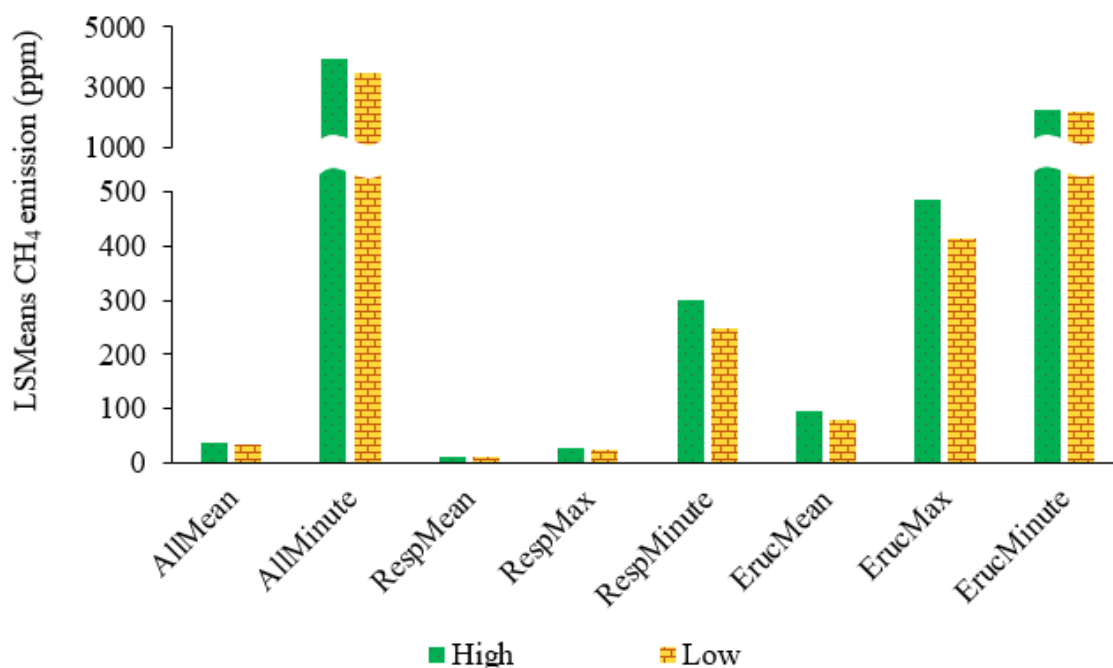


Figure 4.6. Least square means (LSMeans) for the methane traits subjected to a high (High) and a low (Low) neutral detergent fiber intake level.

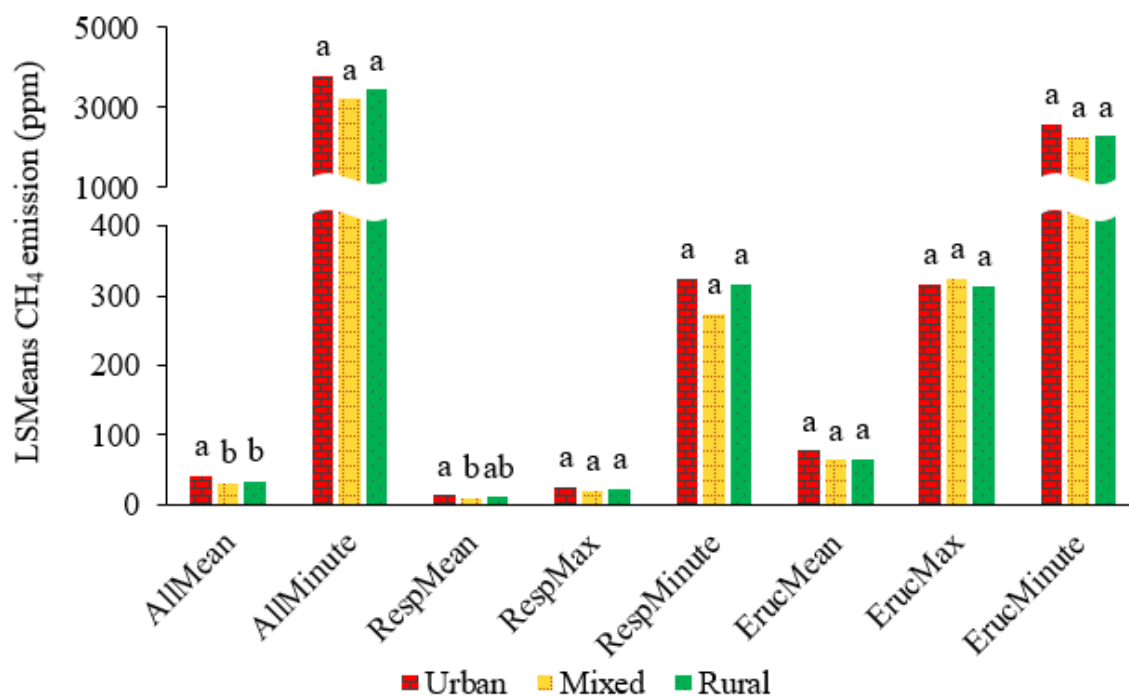


Figure 4.7. Least square means (LSMeans) for the methane traits across three location clusters classified by the survey stratification index. Different letters on bars indicate significant differences (P -value < 0.05).

4. Discussion

4.1. Methane emission traits

In some studies, the spot exhaled air samples were transformed to diurnal CH_4 productions (in g/d) based on a linear regression models (Garnsworthy et al., 2012b; Pszczola et al., 2017), or by considering the ratio between CH_4 and CO_2 concentrations from each visit in automatic

milking systems (Lassen and Løvendahl, 2016). However, the transformations are only accurate for constant CH₄ and CO₂ concentrations throughout the testing day, and for climatic conditions at 25 °C with 1 atmosphere (101.3 kPa) pressure (the density of CH₄ is 665.7 mg/L under these conditions). These assumptions can easily be violated because the CH₄ and CO₂ concentrations definitively vary during the day (van Engelen et al., 2018), and temperature and pressure also differ across visits and farms. Therefore, only the CH₄ concentration as measured by the LMD, in parts per million (ppm), was used in the present study. This information can reflect relative differences in CH₄ concentrations between farms applying different dairy cattle management systems along the rural-urban gradient in Bangalore.

Van Engelen et al. (2018) measured CH₄ concentrations from 1,508 Dutch Holstein Friesian cows with infrared sensors installed in automatic milking systems and reported that the mean CH₄ concentration per visit ranged between 11 and 2,073 ppm (mean = 254 ppm). In comparison, AllMean obtained from LMD ranged from 8.25 to 351.75 ppm (mean = 42.85 ppm), which is significantly lower than AllMean reported by van Engelen et al. (2018). However, CH₄ concentrations, including all, respiration, and eructation CH₄, from steers measured with an LMD (Ricci et al., 2014) are comparable to our findings. The methods applied for measuring CH₄ output can be a reason behind the varying CH₄ emission values. Differences between seasons, sheds and diets contribute to further variations in CH₄ and impede comparison of data from different studies (Ngwabie et al., 2009).

4.2. Body weight and milk yield

Given the diversity in body size and weight of the sampled genotypes in this study, the positive correlation between MY and BW (Berry et al., 2007) would overemphasize breed differences in MY. Also, the imbalanced numbers of cows of different genotypes biased the analysis of MY effects on CH₄ concentration. Therefore, MY was corrected and expressed in liters per kg BW. In this study, AllMean is used as the reference value to represent a full exhalation–inhalation cycle (Chagunda et al., 2009b; Garnsworthy et al., 2019). The positive regression coefficient of 0.03 for MY/BW (*P*-value < 0.05) agrees with the findings from Garnsworthy et al. (2012b), who found that CH₄ emissions were positively associated with MY. Cows on urban dairy farms had higher MY and BW than cows on mixed and rural farms. Usually, CH₄ per unit of product, i.e. liter of milk, decreases with increasing MY (Chagunda et al., 2009a; Kirchgessner et al., 1991). When considering AllMean to calculate CH₄ per liter of milk, urban areas still showed the highest CH₄ emission (4.62 ppm/l) followed by rural areas (3.92 ppm/l), while mixed areas had the lowest CH₄ emission per liter of milk (3.88 ppm/l). In this regard,

farms in the mixed areas seemed to be more efficient in terms of methane output per unit of product (liter of milk).

4.3. Time interval from feeding to CH₄ recording

In line with the decreasing CH₄ concentrations with the increasing time interval from feeding to CH₄ recording reported by Chagunda (2013) and Ricci et al. (2014), the time interval from feeding to CH₄ recording always had negative regression coefficients for all CH₄ traits. Actually, CH₄ emissions are higher after feeding when more substrate is available for rumen fermentation and more hydrogen is available for methanogenesis (Basarab et al., 2013) and it decreases over time when the substrate (feed) is degrading and undegraded feed is getting scarce.

4.4. Breed

Swamy and Bhattacharya (2006) stated that Indian indigenous cattle had higher CH₄ emission per unit of milk than crossbred cattle. In the present study, native cows had highest CH₄ emissions for AllMean, AllMinute and ErucMax (Table 4.4), although no significant difference to the other breeds existed. Chagunda et al. (2009a) compared cattle enteric CH₄ between groups of low and high genetic merit for milk fat and protein content in combination with low and high forage intake and concluded that breeding for cows with high genetic merit was associated with a decrease in enteric CH₄ emissions per liter of milk. However, the number of native cows in our sample was very small (n=13) compared to exotic (n=448) and crossbred cows (n=285) since native cows are not primarily used for dairy production but for fieldwork. In urban areas, since there is no field to cultivate, the native cows are used as dairy cows. For this reason, we found a higher percentage of native dairy cows in urban and mixed than in rural areas (Table 4.1).

4.5. Heat stress and measurement location

Heat stress is associated with a reduction of the dry matter intake in ruminants (Rhoads et al., 2009) and consequently leads to a reduction in enteric CH₄ emissions (Hammond et al., 2009). Even though in this study there was no significant impact of THI on AllMean (Table 4.4), a decrease in emissions of all CH₄ traits was associated with increasing THI. Along the rural-urban gradient, THI on farms located in rural areas (72.85) was higher than on farms in the remaining areas, i.e. urban (70.19) and mixed (69.94). In urban areas, due to the building density, cattle are predominantly kept in the basement or ground floor or in front of the house

under a well-insulated roof. In rural areas, only a simple wooden structure with some hay on top is used as a shed, offering little protection against heat stress. In consequence, the increase of THI in rural areas might be due to the scarcity of shadow and heat insulation.

Independent of the rural-urban gradient, highest CH₄ concentrations were measured in the indoor barns, followed by the half-outdoor and the outdoor sheds (Figure 4.5). As stated above, in the densely populated urban areas, limited space is available for cattle, forcing farmers to keep their cows indoors. In consequence, 46.07% of the CH₄ measurements in urban areas were conducted indoors, compared to 22.59% of indoor CH₄ measurements in rural areas (Table 4.1). According to Ngwabie et al. (2011), indoor air temperature is negatively correlated with daily CH₄ emissions, because with the higher temperatures that prevail in (poorly ventilated) indoor conditions the feed intake decreases, resulting in lower CH₄ emissions. However, in our study, the highest THI was found for half-outdoor locations (72.70) which were the most common in rural areas (Table 4.1). Besides, Ngwabie et al. (2009) also showed that CH₄ concentrations in indoor barns were higher than in outdoor barns and that ventilation rate had an inverse linear relationship to the CH₄ concentrations. Thus, the ventilation determined by shed type has more influence on measured CH₄ emissions relative to dry matter intake than the THI.

4.6. Pasture access and fiber intake

The main factor driving CH₄ production is feed intake. Nevertheless, reducing intake has not been considered as a strategy of CH₄ reduction because of concerns in decreasing animal productivity (Alford et al., 2006). However, modulating fiber content in diets and grazing management have been successfully used as mitigation strategies (Hammond et al., 2016; A. Hristov et al., 2013). Grazing strategies employed to mitigate CH₄ emissions are often related to forage quality, which means type and maturity of the fodder. Thus, feeding higher quality forage facilitates CH₄ mitigation (A. N. Hristov et al., 2013).

Pasture-based dairy farming systems and confinement systems differ in the amount of CH₄ produced (O'Brien et al., 2014). In a tropical environment, the access of cows to pasture changes with season as well as with farm location, cow breed, lactation stage and health status of the cow. In Bangalore, more than half of the cows (53.41%) had access to pasture, although the percentage of pasture access decreased in the dry season when the availability of vegetation declined. Lower CH₄ concentrations were determined in cattle with pasture access, which accounted for 70.68% of cows in urban areas (Table 4.1). Access to high quality pasture ensures lower CH₄ emissions from grazing animals (Robertson and Waghorn, 2002), but due to the

increasing population size and build-up density, the quality of inner-urban pasturing sites (vacant building plots, organic waste dumps, roadsides) in Bangalore was very variable. In urban areas, local shops and neighbors also supply farms with organic waste (vegetable and fruit peels) as cow feed (Reichenbach, 2020). In mixed and rural areas, cows mostly received elephant grass, maize stover and finger millet straw, as well as concentrate feed. In the nutrition subsample, we used the quantitative threshold of 94 g NDF/kg MW to separate fiber intake levels - the qualitative equivalent of this threshold 600 g NDF/kg DM. Similar diets, in terms of NDF concentration, were also reported by Ali et al. (2019) for heifers in Kenya (ca. 700 g NDF/kg DM) although recommendations for the diets of high-producing dairy cows in peak lactation range from 250 to 400 g NDF/kg DM (Mertens, 1994; National Research Council (NRC), 1989; Standing Committee of Agriculture (SCA), 1990). However, no impact of the intake level of fiber (low *versus* high NDF) on CH₄ concentration was detected in our study, even though ruminants emit more CH₄ when ingesting fibrous diet (Moe and Tyrrell, 1979).

4.7. Rural-urban gradient

Results from the present study unveiled impacts of SSI on CH₄ emission. Although the SSI was constructed based on spatial information on a neighborhood's building density and distance to the city center (Hoffmann et al., 2017), it was also able to capture differences in management on the dairy farms. Compared to mixed and rural areas, higher CH₄ concentrations were found for cows kept in urban areas. Urban dairy production is common in India (Prasad et al., 2019) because of the strong market demand for milk products and higher milk prices in urban areas (Pinto et al., 2020). Furthermore, due to Indian culture and tradition, cattle are endeared and respected animals and are commonly allowed to graze or roam freely around in the whole city where they are often able to consume good quality vegetable residues from dumpsites. Access to quality pasture is associated with a decrease in CH₄ emissions (A. Hristov et al., 2013), thus, the higher accessibility to pasture in the mixed areas along with their highest percentage of outdoor locations may explain the lower CH₄ emission in mixed as compared with urban areas. In rural areas, apart from a higher number of outdoor measurement locations, the low productivity (MY) of the cattle and the higher THI might contribute to lower CH₄ emissions than in urban areas.

These examples demonstrate that social-ecological conditions influence farm management of dairy cattle along the rural-urban gradient in Bangalore and lead to variations in CH₄ emissions. On one hand, in urban areas, dairy cows benefited from better management and high quality pasture thus having a higher MY and BW (Reichenbach, 2020). On the other hand, urban dairy

farms are characterized by indoor sheds, lower THI, less heat stress, and improved hygienic and health conditions (Pinto et al., 2020). Although these conditions generally reduce CH₄ emissions, LMD spot measurements in urban locations yielded the highest values. Here, the measurement location type (poorly ventilated indoor barn with higher background CH₄ concentration *versus* well ventilated outdoor barn with lower background CH₄ concentration) may have overridden the positive effects of an improved cattle management in urban areas. In our study, we considered THI (which takes into account temperature and relative humidity) as an environmental factor that may influence the CH₄ measurement. However, Chagunda et al. (2013) additionally considered atmospheric pressure, wind speed and wind direction to correct outdoor LMD emissions. Thus, the CH₄ concentration determined in outdoor locations of Bangalore may not represent the real values and rather underestimate these due to ignorance of the mentioned atmospheric variables.

The impact of a rural-urban gradient on CH₄ emissions displays the relevance of the underlying factors, namely herd management and environmental conditions, as shaped by the (progressing) city environment. An index summarizing those factors, such as, the SSI, can therefore be used as a social-environmental feature in which human/management effects and environmental effects (e.g. THI) are expressed together.

5. Conclusions

This study is a pioneer in the use of the LMD for on-farm methane emission measurements of dairy cattle in an urbanizing environment. The LMD technique showed variations in the CH₄ emissions along the rural-urban gradient of Bangalore. The additional consideration of SSI in CH₄ emission contributed to capturing variations in dairy husbandry systems that are variations associated with social-ecological conditions. Except for the fiber content in the diet (NDF), which was inconclusive due to the small sample size, the remaining effects, i.e. milk yield, body weight, the time interval between feeding and CH₄ measurement, pasture access and SSI cluster, as well as THI and measurement location (type of shed), significantly affected CH₄ emissions. Higher concentrations of CH₄ were associated with cows in urban areas with higher milk yield, kept in poorly ventilated indoor sheds but lower THI. Outdoor measurement locations and the high heterogeneity of farm management, worked in favor of the rural farms' CH₄ emissions. Variations in individual CH₄ emissions indicate potential for reduction, e.g. via breeding and feeding strategies. Nevertheless, some disturbing factors as identified in the present study suggest a standardization and improvements of LMD-based CH₄ measurements.

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CHAPTER 5

Gastrointestinal nematode and *Eimeria* spp. infections in dairy cattle along a rural-urban gradient

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1. Introduction

Gastrointestinal nematode (GIN) infections negatively impact productivity, fertility and weight gain in cattle, resulting in considerable economic losses (Charlier et al., 2009). Moreover, infections with protozoan parasites *Eimeria* spp. can cause watery diarrhea, poor appetite, changes in behavior and reduced growth rate in cattle of all ages (Hooshmand-Rad et al., 1994; Sudhakara Reddy et al., 2015). Host-related factors which contribute to GIN and *Eimeria* spp. infections include genetic aspects (e.g. breed variations), the age and the immune response of the host (Gasbarre et al., 2001; Passafaro et al., 2015; Peña et al., 2000). Furthermore, environmental factors (e.g. farm management, habitat) were associated with endoparasite prevalence and virulence (Gillandt et al., 2018; Singh et al., 2017). Future climate scenarios as a result of climate change will directly (e.g. through the variation of temperature and humidity) and indirectly (e.g. through changes in farm management) affect GIN infection dynamics in livestock (Verschave et al., 2016).

In developing countries, rural farmers often do not have the resources to cope with adverse climatic conditions pushing them to migrate to urban areas (Henderson et al., 2017). Mondal et al. (2000) indicated a strong effect of urbanization on the GIN prevalence in ruminants, due to the intensification of grazing in small areas, implying forage contamination with feces due to a high animal density. Especially in urban areas, disease susceptibility and infection intensity are influenced by human-animal cohabitation (Coles, 2002; Ivemeyer et al., 2011). Close human-human cohabitation improves social relations and the availability of inputs (e.g. veterinary services) which might be the basis for the establishment of an efficient farm management that reduces disease infections (Manivannanan and Tripathi, 2007). The combination of social and environmental effects within the so-called social-ecological system's framework already contributed to an improved understanding of bacterial disease infection chains (e.g. mastitis in dairy cattle), also from an economic perspective (Joshi and Gokhale, 2006b). Rising megacities are hotspots of complex and dynamic social-ecological systems, because they are very vulnerable to environmental and anthropogenic hazards, and their socio-economic components are more diverse than those of smaller urban areas (Kraas, 2007).

The dynamics and infection rates of GIN are well-studied in the Northern states of India (Bandyopadhyay et al., 2010; Gupta et al., 2012; Jithendran and Bhat, 1999; Marskole et al., 2016; Wadhwa et al., 2011), whereas only a limited number of rural studies are available for the Southern states. This underlines the importance of analyzing GIN and *Eimeria* spp. infection rates in Southern India in general and in the urbanizing space of Bangalore in particular. A high

prevalence up to 48% was reported for GIN infections in cattle in Northern India, depending on geographical region and season of the year (Choubisa and Jaroli, 2013). For *Eimeria* spp., Laha et al. (2013) identified 28% of cattle with infections in the state of Meghalaya in North-Eastern India. Krishna Murthy and Souza (2016) reported a prevalence of 41.3% for GIN and of 26.9% for *Eimeria* spp. in the Bangalore district of Karnataka. GIN and *Eimeria* spp. infections were studied in dependency of urban to (peri-)urban gradients for goats and cattle in Africa (Kanyari et al., 2010; Mhoma et al., 2011), and in dependency of rural to urban gradients for wildlife species in India and the United Kingdom (Debenham et al., 2017; Richards et al., 1995). Mhoma et al. (2011) identified higher GIN prevalence in goats in urban (68.9%) compared with (peri-)urban areas (47.3%) in Mwanza City, Tanzania. The differences point to the importance of space constrains and animal husbandry practices in urban areas for increased incidence of GIN infections.

Bangalore is characterized by rapid population growth, where population doubled in only 15 years and will reach to 16 million inhabitants in 2021 (Groupe SCE India, 2016). Urban and (peri-)urban farmers from Bangalore contribute to the city milk supply by keeping dairy cattle in close to distance their living space (Prasad et al., 2019). Hence, Bangalore is the ideal research location to study endoparasite prevalence rates in cattle along a rural-urban gradient. Therefore, the present study aimed at determining the effect of urbanization on endoparasite infections of dairy cattle in Bangalore, an emerging megacity in South India. Specifically, the objectives of the present study were to (i) investigate the prevalence of GIN and *Eimeria* spp. infections in dairy cattle of different ages in South India and (ii) infer the effect of social-ecological components on the prevalence and egg/oocyst counts for GIN and *Eimeria* spp. in dairy cattle along the rural-urban gradient in the emerging megacity Bangalore in South India.

2. Materials and Methods

2.1 Study area

The study was carried out in the megacity Bangalore, located in the southern Indian state of Karnataka at 12.97° northern latitude and 77.59° eastern longitude, 920 meters above sea level. The climate is of tropical savannah type with the two main periods dry and humid. The humid monsoon period includes the months from June to September. According to the weather station data of the University of Agricultural Sciences Bangalore for the time-period from 2013 to 2017, the wettest month is September with an average total rainfall of 214.5 mm. The driest month is February with an average of only 0.44 mm rainfall. The months October and

November (autumn season) are in-between the humid and dry period. Two seasons can be distinguished in the dry period: a winter season including December, January and February (daily temperatures between 14.1 and 31.2 °C) and a summer season including March, April, and May (daily temperatures between 18.2 and 35.8 °C).

2.2 Farms and animals

Dairy farms were sampled from 30 different villages located along a northern and southern transect in Bangalore (Figure 5.1). Both transects cut through the city following a survey stratification index (SSI). The SSI was developed by Hoffmann et al. (2017) in order to characterize the rural-urban interface of Bangalore. The SSI considers the built-up density (houses and infrastructures) and the distance to the city center of each location to classify them as “urban” (SSI < 0.3), “rural” (SSI > 0.5), and “(peri-)urban” or “mixed” (SSI: 0.3 - 0.5). A two-step approach was applied to select 101 dairy farms. In a first step, villages were selected semi-randomly, considering pre-defined percentages per SSI. In a second step, 20 to 30% dairy cow herds were randomly selected per village (300 herds), based on the latest vaccination list for foot-and-mouth disease. Only dairy cow herds with two or more dairy cows were sampled. The original 300 dairy cow herds were reduced to 101 herds after clustering them into four groups based on geographic coordinates for herd location, feeding strategies and predominant cattle genotypes in the herd. Of the 101 dairy farms, 17 were located in the urban area, 29 in the mixed area and 55 in the rural areas. The herd sizes ranged from 2 to 11 cattle. The mean herd size in comprised 5.0 cattle in urban, 3.7 cattle in mixed and 3.5 cattle in rural areas.

The study population included 305 lactating cows, 85 dry cows, 63 heifers (female aged ≥ 13 months until they deliver their first calf) and 55 calves (males and females aged 0 to 12 months). Since some individuals changed physiological status (e.g. from calf to heifer) during the sample collection, the total number of surveyed animals equaled 441. Breeds present on the farms included exotic breeds (Holstein Friesian and Jersey, $n = 285$), native breeds (Zebu cattle e.g. Hallikar, $n = 18$) and crossbreeds (exotic x native, $n = 138$). Access to pasture (yes/no) was recorded for each animal at every visit.

2.3 Fecal sampling and examination

Each farm was visited three times between June 2017 and April 2018 in a four-month interval. Throughout the sampling period, 726 fecal samples were rectally taken from the 441 cattle, and stored in dry and clean polythene bags. Fecal samples were instantly kept in a cooling box until they were stored in a fridge and examined within 24 hours after collection. We used a modified

McMaster technique according to Thienpont et al. (1979) with 4g feces and saturated NaCl as flotation solution to count the number of GIN eggs per gram feces (EpG) and *Eimeria* spp. oocysts per gram feces (OpG). The sensitivity of the McMaster technique was 50 EpG/OpG. The number of fecal samples per breed, cattle physiological status, season and pasture access in dependency of SSI are given in Table 5.1.

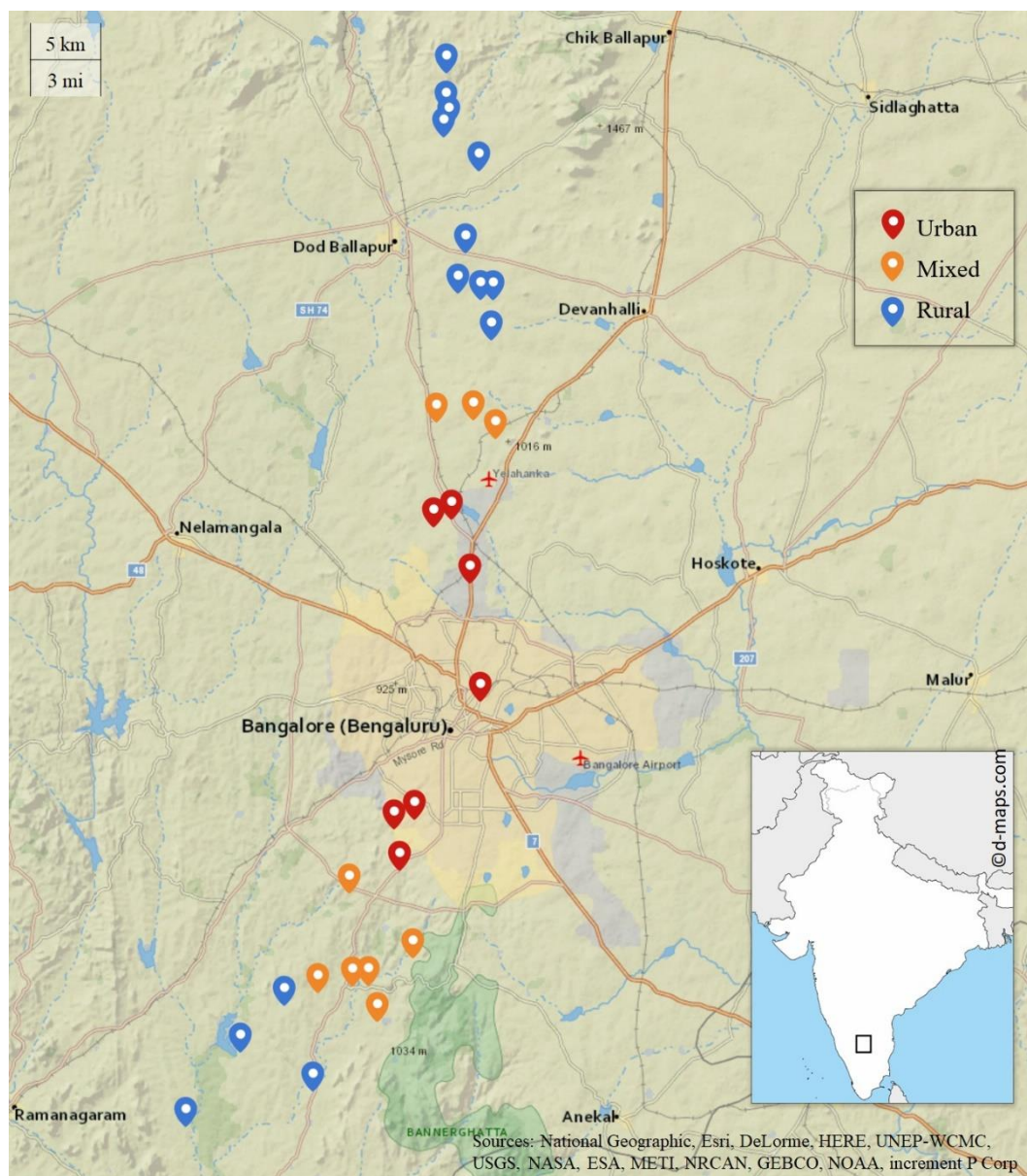


Figure 5.1. Geographic distribution of the 30 villages included in the study.

2.4 Statistical analyses

Eggs and oocysts per gram feces (EpG and OpG) were log-transformed with $\ln(\text{EpG or OpG} + 100)$. Additionally, EpG and OpG were transformed on a y^{λ_i} scale obtained from the Box–Cox transformation, in which $y^{\lambda_i} = (y^{\lambda_i} - 1)/\lambda$, ($\lambda \neq 0$) or $y^{\lambda_i} = \ln(y_i)$, ($\lambda = 0$), in order to achieve a Gaussian-like distribution for subsequent model analyses (da Silva et al., 2011). Furthermore,

EpG and OpG were considered as binary endoparasite data (BinEpG/BinOpG), displaying the presence or absence of endoparasite species. In this regard, cattle were classified based on their infection status as infected (presence of GIN eggs/*Eimeria* spp. oocysts in feces = 1) and non-infected (absence of GIN eggs/*Eimeria* spp. oocysts in feces = 0). Descriptive statistics for egg/oocyst counts and for binomial endoparasite data is given in Table 5.2.

Table 5.1. Descriptive statistics for endoparasite data.

Endoparasite parameter	Observations	Farms	Cattle	Min	Max	Mean	SD
BinEpG	726	101	441	0	1	0.21	0.41
BinOpG	726	101	441	0	1	0.33	0.47
EpG	726	101	441	0	1900	65.01	196.63
OpG	726	101	441	0	15000	170.50	1031.50

Min = Minimum value; Max = Maximum value; SD = Standard deviation; BinEpG = Binary defined EpG (EpG = 0 classified as negative for gastrointestinal nematodes; EpG = 1 classified as positive for gastrointestinal nematodes); BinOpG = Binary defined OpG (OpG = 0 classified as negative for *Eimeria* spp.; OpG = 1 classified as positive for *Eimeria* spp.); EpG = Eggs per gram feces for gastrointestinal nematodes; OpG = Oocysts per gram feces for *Eimeria* spp.

Statistical analyses were performed using the statistical software R version 3.6.1 (R Core Team, 2019). For all variance analyses, P -values ≤ 0.05 for defined effects were regarded as significant. To assess the influence of SSI and further risk factors on the infection probability for GIN (BinEpG) and *Eimeria* spp. (BinOpG), we applied a generalized linear mixed model (GLMM) with a logit link function as implemented in the glmer function in the lme4 package in R (Bates et al., 2015). The statistical model 1 was:

$$\text{logit}(\pi_{ijklmno}) = \log [\pi_{ijklmno} / (1 - \pi_{ijklmno})] = \varphi + \text{SSI}_i + \text{Breed}_j + \text{Status}_k + \text{Season}_l + \text{Pasture}_m + F_n + C_o \quad [1]$$

where $\text{logit}(\pi_{ijklmno})$ is the probability of a cattle to be infected with GIN (BinEpG) or *Eimeria* spp. (BinOpG); φ is the overall mean effect; SSI_i is the fixed effect of SSI (urban, mixed and rural); Breed_j is the fixed effect of the breed (exotic, native and crossbreed); Status_k is the fixed effect of cattle physiological status (lactating, dry, heifer and calf); Season_l is the fixed effect of parasitological sampling season (monsoon, autumn, winter and summer); Pasture_m is the fixed effect of pasture access (yes or no); F_n is the random farm effect; C_o is the random cattle effect accounting for repeated parasitological measurements.

Table 5.2. Number (No.) and percentage (%) of fecal samples in relation to breed, cattle physiological status, season and pasture access for cattle kept across three survey stratification index (SSI) classes in Bangalore.

SSI class	Breed (No./%)			Cattle physiological status (No./%)				Season (No./%)				Pasture (No./%)		
	No.	Exotic	Crossbreed	Native	Lactating	Dry	Heifer	Calf	Monsoon	Autumn	Winter	Summer	Yes	No
Urban	125	66/52.8	53/42.4	6/4.8	90/72.0	11/8.8	12/9.6	12/9.6	16/12.8	51/40.8	45/36.0	13/10.4	98/78.4	27/21.6
Mixed	186	117/62.9	59/31.7	10/5.4	118/63.4	29/15.6	24/12.9	15/8.1	27/14.5	42/22.6	93/50.0	24/12.9	96/51.6	90/48.4
Rural	415	270/65.1	130/31.3	15/3.6	242/58.3	51/12.9	56/13.5	66/15.9	63/15.2	73/17.6	132/31.8	147/35.4	120/28.9	295/71.1

A linear mixed model (LMM) as implemented in the ‘lmer’ function (Bates et al., 2015) was applied to analyze the effect of SSI and further risk factors on EpG and OpG. The statistical model 2 was:

$$y_{ijklmnop} = \varphi + \text{SSI}_i + \text{Breed}_j + \text{Status}_k + \text{Season}_l + \text{Pasture}_m + F_n + C_o + e_{ijklmnop} \quad [2]$$

where $y_{ijklmnop}$ is the logarithmic transformed egg/oocyst count $\ln(\text{EpG}+100)$ and $\ln(\text{OpG}+100)$; φ is the overall mean effect; $e_{ijklmnop}$ is the random residual effect and further effects as specified in model 1.

Model 2 was applied for log-transformed EpG and OpG, for Box-Cox transformed EpG and OpG and for the untransformed EpG and OpG. The best model results (lowest Akaike information criteria [AIC] and Bayesian information criteria [BIC]) were achieved for the log-transformed EpG/OpG in all runs. We tested interaction terms between SSI and other fixed effects in model 1 and 2, but the model results revealed no significances ($P > 0.05$). Hence, no interaction term was included in the final mixed model analyses. Fixed effects in the models were tested for significance by stepwise selection. We selected the models with the smallest AIC and BIC as the best model. For model validation, we checked the normal distribution of residuals by histograms. Least-squares means (LSMeans) for $\ln(\text{EpG}+100)$ and $\ln(\text{OpG}+100)$ were back-transformed automatically to the original scale in R.

3. Results

3.1 Farm-level prevalence

Gastrointestinal nematode infections occurred in 80.2% (81/101) of all farms. The GIN prevalence within farms (farm-level prevalence) ranged from 0.0 to 100.0% with a mean farm-level prevalence of 31.2%. The predominant GIN species were Trichostrongylidae and other Strongylida (Chabertiidae and Ancylostomatidae), which were detected in 79.2% (80/101) of the farms. We identified *Strongyloides papillosus* in 7.9% (8/101) and *Trichuris* spp. in 2.0% (2/101) of all farms. *Eimeria* spp. infections were present in 48.5% (49/101) of all farms. As for GIN, farm-level prevalence for *Eimeria* spp. ranged from 0.0 to 100.0% with a mean farm-prevalence of 15.8%. Co-infections of GIN and *Eimeria* spp. were noticed in 42.6% (43/101) of the farms. Mono-infections (sole presence of GIN or *Eimeria* spp. within farm) were observed in 42.6% (43/101) of all farms with 37.6% (38/101) of farms presenting GIN infections and 4.9% (5/101) of farms presenting *Eimeria* spp. infections exclusively. The remaining 14.8% (15/101) farms were non-infected.

3.2 Individual cattle prevalence and endoparasite co-infections

In total, 43.9% (319/726) of all fecal samples were positive for endoparasite infections (GIN and/or *Eimeria* spp.). Among the 319 positive samples, 52.7% (168/319) showed positive EpG values of ≥ 50 ; 23.8% (76/319) showed positive OpG values of ≥ 50 and 23.5% (75/319) showed positive co-infection of EpG and OpG values of ≥ 50 . Lactating and dry cows represented a lower mean EpG (39.0 and 38.5, respectively) compared to calves and heifers (215.0 and 66.8, respectively). Accordingly, the mean OpG was lower in lactating and dry cows (14.6 and 20.3, respectively) compared with calves and heifers (1121.5 and 120.6, respectively; Table 5.3). Strongylid eggs were detected in 33.1% (240/726) of all samples (Table 5.4). We identified *Strongyloides papillosus* in 1.4% (10/726) and *Trichuris* spp. in 0.7% (2/726) of all samples. *Eimeria* spp. infections were present in 20.8% (151/726) of all samples.

Regarding the 441 individual dairy cattle, GIN infections occurred in 41.7% (184/441). Strongylid eggs were detected in 41.5% (183/441) of all dairy cattle. We identified *Strongyloides papillosus* in 2.3% (10/441) and *Trichuris* spp. in 0.4% (2/441) of all dairy cattle. Coccidian oocysts were present in 25.6% (113/441) of all dairy cattle. Co-infections of GIN and *Eimeria* spp. were detected in 13.6% (60/441), while 37.9% (167/441) of all dairy cattle were infected with GIN or *Eimeria* spp. solely. The remaining 48.5% (214/441) were non-infected.

3.3 Individual cattle prevalence along a survey stratification index (SSI)

Strongylid eggs were detected in 39.2% (49/125) of urban samples, in 34.2% (142/415) of rural samples and in 26.3% (49/186) of fecal samples from mixed areas (Table 5.4). *Strongyloides papillosus* was detected in 2.4% (3/125) of fecal samples from urban areas, in 2.2% (4/186) of samples from mixed areas and in 0.7% (3/415) of rural samples (Table 5.4). We observed *Trichuris* spp. in 0.8% (1/125) of urban samples, in 0.5% (1/186) of samples from mixed areas and in 0% (0/415) of rural samples (Table 5.4). Co-infections of GIN and *Eimeria* spp. were detected in 10.3% (75/726) of all samples. Co-infection rates of GIN and *Eimeria* spp. were higher in rural areas with 14.9% (62/415), followed by urban with 6.4% (8/125) and mixed areas with 2.7% (5/186) co-infected samples (Figure 5.2).

Table 5.3. Total number of fecal samples, prevalence [number (No.) and % of positive samples (%)], mean eggs per gram feces (EpG) for gastrointestinal nematodes (GIN) and mean oocysts per gram feces (OpG) for *Eimeria* spp. with regard to the cattle physiological status.

Cattle physiological status	No. of tested samples	GIN					<i>Eimeria</i> spp.				
		Positive samples (No./%)	Mean EpG	SD	Min	Max	Positive samples (No./%)	Mean OpG	SD	Min	Max
Lactating	450	127/28.2	39.0	130.51	0	1850	50/11.1	14.6	65.94	0	800
Dry	91	21/23.1	38.5	132.09	0	1100	12/13.2	20.3	81.64	0	550
Heifer	92	42/45.6	66.8	147.18	0	1100	38/41.3	120.6	330.51	0	2700
Calf	93	53/57.0	215.0	395.34	0	1900	51/54.8	1121.5	2681.57	0	15000
Total	726	243/33.5	65.0	196.63	0	1900	151/20.8	170.5	1031.49	0	15000

SD = Standard deviation; Min = Minimum value; Max = Maximum value

Table 5.4. Total number of fecal samples and prevalence [number (No.) and % of positive samples (%)] for the different endoparasite species within the survey stratification index (SSI) classes.

Endoparasite species	SSI									Total positive samples (No./%)
	Urban			Mixed			Rural			
	No. of tested samples	Positive samples (No./%)	Mean EpG/OpG	No. of tested samples	Positive samples (No./%)	Mean EpG/OpG	No. of tested samples	Positive samples (No./%)	Mean EpG/OpG	
Trichostrongylidae/Stongylida	125	49/39.2	69.6	186	49/26.3	41.7	415	142/34.2	68.1	240/33.1
<i>Strongyloides papillosus</i>	125	3/2.4	2.8	186	4/2.2	2.4	415	3/0.7	3.7	10/1.4
<i>Trichuris</i> spp.	125	1/0.8	0.4	186	1/0.5	0.5	415	0/0.0	0.0	2/0.7
<i>Eimeria</i> spp.	125	10/8.0	66.8	186	15/8.1	7.8	415	126/30.4	274.7	151/20.8

EpG = Eggs per gram feces for gastrointestinal nematodes; OpG = Oocysts per gram feces for *Eimeria* spp

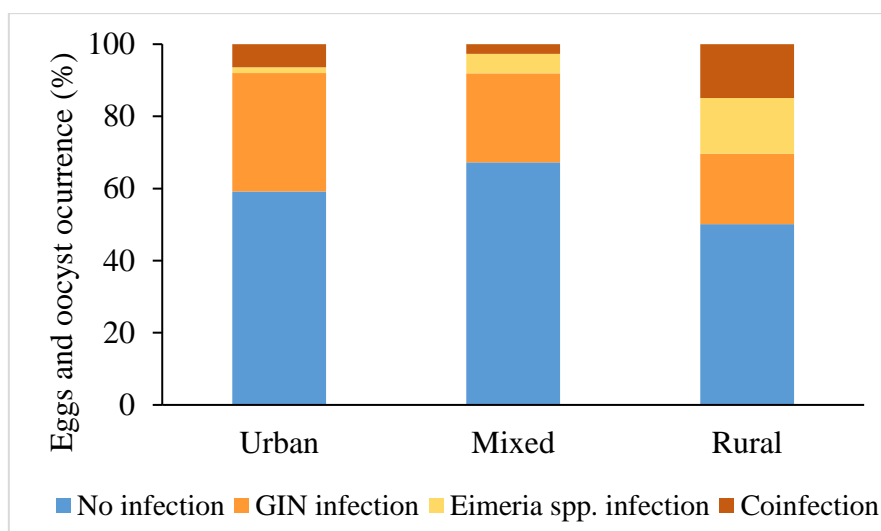


Figure 5.2. Occurrence of endoparasite infections (%) by infection type (no infection, mono-infection for gastrointestinal nematodes or *Eimeria* spp. and co-infection for gastrointestinal nematodes and *Eimeria* spp.) within survey stratification index (SSI) classes.

3.4 Impact of survey stratification index (SSI) on endoparasite infections

Results for the test of significance for fixed effects (Type III test of fixed effect) and LSMeans for the four endoparasite infection traits BinEpG, BinOpG, EpG and OpG are given in Table 5.5. The SSI effect significantly influenced the *Eimeria* spp. infection probability (BinOpG) and the oocyst counts (OpG) ($P < 0.001$) LSMeans for BinOpG were significantly higher in rural areas (32.6%) in comparison to mixed areas (9.2%; $P < 0.001$) and urban areas (6.3%; $P = 0.001$) (Figure 5.3). Moreover, the LSMean for OpG was significantly higher in dairy cattle kept in rural areas (78.8) compared to dairy cattle kept in urban (37.4; $P = 0.003$) or mixed areas (34.2; $P < 0.001$) (Figure 5.4). Infection probabilities for GIN (BinEpG) in different areas ranged from 32.9 to 42.5% with the lowest infection probability in mixed areas and the highest infection probability in urban areas ($P = 0.387$). For EpG, LSMean was highest in rural areas (42.7) compared to urban (41.9) and mixed areas (36.2), but differences between SSI classes were not statistically significant ($P = 0.731$).

3.5 Impact of breed on endoparasite infections

The breed had no significant effect on endoparasite infection parameters (Table 5.5). The LSMeans for BinEpG were highest for the native breed (38.8%) followed by exotic (38.6%) and crossbred (37.4%; $P = 0.968$). Regarding BinOpG, LSMeans were higher for exotic (16.0%) compared to the crossbred (15.2%) and native (8.8%) breed ($P = 0.631$). Although native dairy cattle had the lowest LSMean for BinOpG, they represented the highest LSMean for OpG (63.9). Similarly, crossbreeds showed the lowest LSMean for BinEpG, but the highest

LSMean for EpG (41.9; $P = 0.946$). Generally, the native breed had the lowest LSMean for EpG (37.1), and the crossbreeds had the lowest LSMean for OpG (34.5; $P = 0.079$).

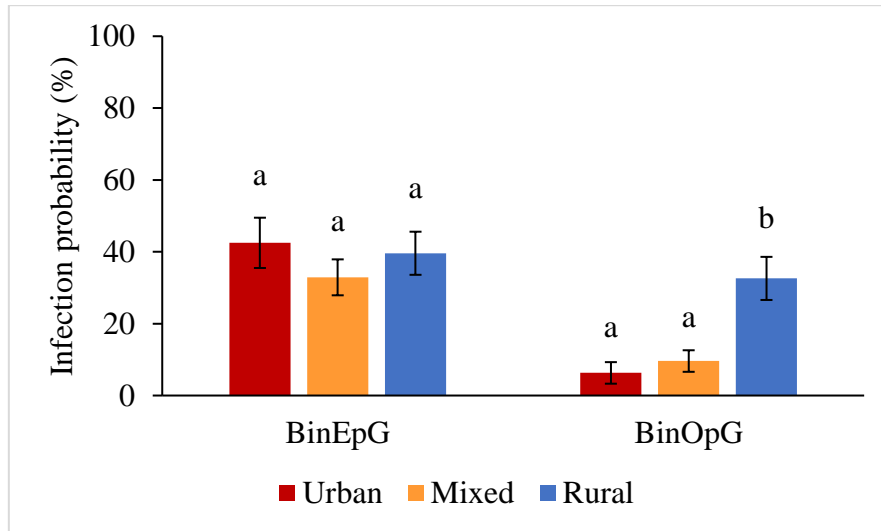


Figure 5.3. Least square means with corresponding standard errors for the infection probability of gastrointestinal nematodes (BinEpG) and *Eimeria* spp. (BinOpG) within the survey stratification index (SSI) classes. Different letters on bars indicate significant difference (P -value ≤ 0.05).

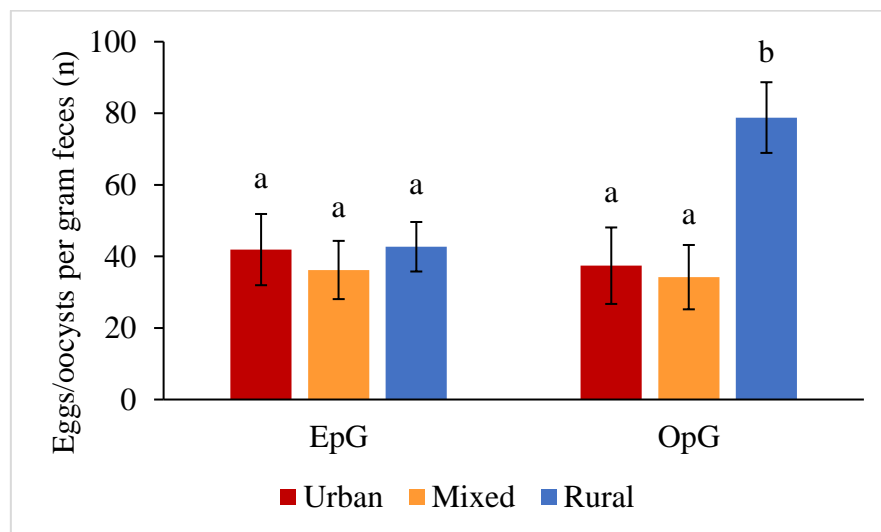


Figure 5.4. Least square means with corresponding standard errors for egg per gram feces for gastrointestinal nematodes (EpG) and oocysts per gram feces for *Eimeria* spp. (OpG) within the survey stratification index (SSI) classes. Different letters on bars indicate significant differences (P -value ≤ 0.05).

Table 5.5. Least-squares means with corresponding standard error (\pm SE) and *P-values* (Type III test of fixed effect) for the infection probability of gastrointestinal nematodes (BinEpG) and *Eimeria* spp. (BinOpG), for eggs per gram feces of gastrointestinal nematodes (EpG) and for oocysts per gram feces of *Eimeria* spp. (OpG) for fixed effect classes included in the model 1 and 2.

Fixed effects	Effect class	BinEpG	<i>P-value</i>	BinOpG	<i>P-value</i>	EpG	<i>P-value</i>	OpG	<i>P-value</i>
SSI			0.387		$\leq 10^{-4}$		0.731		$\leq 10^{-4}$
	Urban	0.42 \pm 0.07 ^a		0.06 \pm 0.03 ^a		41.90 \pm 9.95 ^a		37.39 \pm 10.69 ^a	
	Mixed	0.33 \pm 0.05 ^a		0.09 \pm 0.03 ^a		36.21 \pm 8.14 ^a		34.22 \pm 8.99 ^a	
	Rural	0.39 \pm 0.06 ^a		0.32 \pm 0.06 ^b		42.70 \pm 6.92 ^a		78.84 \pm 9.88 ^b	
Breed			0.968		0.631		0.946		0.079
	Exotic	0.39 \pm 0.04 ^a		0.16 \pm 0.03 ^a		41.74 \pm 5.28 ^a		49.52 \pm 6.30 ^a	
	Crossbreed	0.37 \pm 0.05 ^a		0.15 \pm 0.03 ^a		41.88 \pm 6.29 ^a		34.53 \pm 6.83 ^a	
	Native	0.39 \pm 0.11 ^a		0.09 \pm 0.06 ^a		37.14 \pm 13.94 ^a		63.95 \pm 20.15 ^a	
Status			$\leq 10^{-4}$		$\leq 10^{-4}$		$\leq 10^{-4}$		$\leq 10^{-4}$
	Lactating	0.28 \pm 0.04 ^a		0.05 \pm 0.02 ^a		19.45 \pm 5.24 ^a		7.47 \pm 5.51 ^a	
	Dry	0.23 \pm 0.05 ^a		0.05 \pm 0.02 ^a		18.87 \pm 7.27 ^{ab}		7.60 \pm 7.92 ^a	
	Heifer	0.48 \pm 0.07 ^b		0.25 \pm 0.07 ^b		42.58 \pm 9.56 ^b		48.08 \pm 11.96 ^b	
	Calf	0.58 \pm 0.07 ^b		0.33 \pm 0.08 ^b		91.06 \pm 13.18 ^c		186.66 \pm 23.52 ^c	
Season			0.221		0.276		0.095		0.004
	Monsoon	0.45 \pm 0.07 ^a		0.16 \pm 0.05 ^a		39.96 \pm 8.78 ^a		64.93 \pm 12.26 ^a	
	Autumn	0.40 \pm 0.06 ^a		0.15 \pm 0.05 ^a		50.97 \pm 7.96 ^a		59.35 \pm 9.90 ^a	
	Winter	0.37 \pm 0.05 ^a		0.10 \pm 0.03 ^a		38.82 \pm 6.91 ^a		41.60 \pm 8.30 ^{ab}	
	Summer	0.31 \pm 0.06 ^a		0.11 \pm 0.04 ^a		31.87 \pm 7.39 ^a		31.91 \pm 8.71 ^b	
Pasture			0.307		0.926		0.922		0.715
	Yes	0.41 \pm 0.05 ^a		0.13 \pm 0.04 ^a		39.91 \pm 6.97 ^a		50.37 \pm 8.62 ^a	
	No	0.36 \pm 0.05 ^a		0.13 \pm 0.04 ^a		40.57 \pm 7.17 ^a		47.35 \pm 8.72 ^a	

SSI = Survey stratification index

Different subscripts indicate significant differences (*P-value* \leq 0.05).

3.6 Impact of cattle physiological status on endoparasite infections

The cattle physiological status significantly influenced all endoparasite infection parameters ($P < 0.001$). We estimated significantly higher LSMMeans for BinEpG for calves (58.1%; $P < 0.001$) and for heifers (48.0%; $P < 0.01$) compared to lactating (28.0%) and dry cows (22.8%) (Table 5.5). Accordingly, LSMMeans for BinOpG were significantly higher for calves (32.9%; $P < 0.001$) and heifers (25.1%; $P < 0.001$) in comparison to lactating cows (4.9%) and dry cows (5.4%) (Table 5.5). The LSMean for EpG was significantly higher for calves (91.1) compared to heifers (42.6; $P = 0.001$), lactating cows (19.4; $P < 0.001$) and dry cows (18.9; $P < 0.001$). However, there was no significant difference between dry cows with heifers ($P = 0.067$) and lactating cows ($P = 0.999$). Similarly, LSMean for OpG was significantly higher for calves (186.7) compared to heifers (48.1; $P < 0.001$), dry cows (7.6; $P < 0.001$) and lactating cows (7.5; $P < 0.001$). No significant difference was found LSMean for OpG between dry and lactating cows ($P = 1$).

3.7 Impact of season on endoparasite infections

The season significantly influenced OpG ($P = 0.004$), while the other infection parameters BinEpG ($P = 0.221$), BinOpG ($P = 0.276$) and EpG ($P = 0.095$) were unaffected (Table 5.5). We estimated significantly lower LSMMeans for OpG in summer (31.9) in comparison to autumn (59.3; $P = 0.02$) and monsoon (64.9; $P = 0.01$). The summer season showed the lowest LSMMeans for BinEpG, EpG and OpG (31.4%; 31.9 and 31.9 respectively). For BinOpG, the lowest LSMMeans were estimated for the winter season (10.0%). Monsoon season showed the highest LSMMeans for BinEpG, BinOpG and OpG (45.1%; 16.3% and 64.9 respectively). For EpG, the highest LSMean was estimated for the autumn season (51.0).

3.8 Impact of pasture access on endoparasite infections

Pasture access was not statistically significant in model 1 and model 2 (Table 5). The LSMean for BinEpG was 41.0% for dairy cattle with access to pasture and 35.6% for dairy cattle without pasture access ($P = 0.307$). For BinOpG, dairy cattle with and without access to pasture showed equal LSMMeans (13.1%; $P = 0.926$). The LSMean for EpG was 39.9 for dairy cattle with pasture access and 40.6 for dairy cattle without access to pasture ($P = 0.922$). In contrast, the average OpG was higher for dairy cattle with access to pasture (50.4) compared to dairy cattle without pasture access (47.3) ($P = 0.715$).

4. Discussion

In the present study, we used the SSI as a novel descriptor of social-ecological effects along a rural-urban gradient in a megacity to investigate their impact on endoparasite infections in dairy cattle. Hoffmann et al. (2017) constructed the SSI based on spatial information on building density and distance to the city center. Hence, the SSI captures social-ecological characteristics that may influence the farm management of dairy cattle along the rural-urban gradient in Bangalore. Social-ecological effects have an important impact on cattle diseases. For example, Mogotsi et al. (2016) described complex social-ecological dynamics in the spread of foot and mouth disease in Botswana. Those dynamics were related to the free movement of livestock, contact between wildlife and livestock, and farmer apathy towards livestock management. Similarly, Keeling et al. (2001) showed that the intensive contact among farmers and the movement of livestock affected the spread of foot and mouth disease in the 2001 outbreak in the United Kingdom. In our study, we hypothesized differences in endoparasite infections along the rural-urban gradient exists since social-ecological components differ along the SSI of Bangalore (Reichenbach, 2020). Based on our results, the SSI significantly influenced *Eimeria* spp. infection probability (BinOpG) and oocyst counts (OpG). *Eimeria* spp. infection probability and OpG were significantly higher in rural areas compared to mixed and urban areas. In contrast, we identified no associations between SSI and GIN infection probability (BinEpG) or GIN egg counts (EpG). Associations between endoparasite infections in ruminants and farm location in urban *versus* (peri-)urban areas were previously reported (Kanyari et al., 2010; Mhoma et al., 2011). However, to the best of our knowledge, no study addressed the dynamics of endoparasite infections along a rural-urban gradient in ruminants. Mhoma et al. (2011) estimated higher prevalences for coccidia and strongyle parasites in goats kept in urban compared to (peri-)urban areas of Mwanza City, Tanzania. The goats mostly roam freely and feed on city garbage dumps where they are overstocking in smaller spaces, favoring coccidia and nematode infections. Accordingly, our study results indicate a higher GIN infection probability and higher EpG in urban compared to mixed areas ($P > 0.05$). Indian cattle are allowed to freely roam the city due to the social and cultural sacred status of the cow. Nevertheless, overstocking is a major problem in India, contributing to increased availability of infective GIN larvae on pasture (Marskole et al., 2016). Based on personal communications with farmers, pasture overstocking is more severe in urban areas since land availability in these areas is scarce and grazing is a more common practice. In our study, pasture access was more frequent in urban (78.4%) areas where GIN prevalence was higher compared with mixed and rural areas, supporting the theory of overstocked pastures in urban areas. Richards et al. (1995)

studied differences in intestinal helminth infections in urban and rural foxes from southern England and in agreement with our findings, they identified parasite species-specific effects between habitats (urban *versus* rural). Interestingly, rural foxes were significantly higher infected with *Toxocara canis*, *Uncinaria stenocephala* and *Taenia pisiformis* compared with urban foxes, while urban foxes were significantly higher infected with *Dipylidium caninum*, *Brachylaima recurve* and *Cryptocotyle lingua* compared with rural foxes. No significant differences were identified between urban and rural foxes for *Toxascaris leonina* and *Taenia hydatigena* infections. Variations in the diet were given as a possible explanation, since rural foxes, for example, are more exposed to *Taenia pisiformis* cysticerci which develop in infected rabbits as an intermediate host (Richards et al., 1995).

Feeding strategies differ between rural and urban areas in Bangalore (Reichenbach, 2020) with a better body condition in urban compared to rural farms (Pinto et al., 2020). Hence, poor body condition might result in impaired resistance (Kambarage et al., 1996; Telila et al., 2014), explaining the high LSMeans for EpG/OpG in rural areas. Moreover, urbanization brings wildlife, livestock and humans closer together, favoring zoonotic parasitic infections induced by human nutritional factors (Gordon et al., 2016). Hence, follow-up studies in cattle should focus on effects of rural-urban gradients on zoonotic parasitic infections in Bangalore. For example, the zoonotic agent *Fasciola* spp. is widely prevalent in Indian cattle (Gupta and Singh, 2002) and was reported in humans (Tandon et al., 2015), raising the interest to study effects of urbanization on food-borne trematodiasis.

For *Eimeria* spp. infections, Rehman et al. (2011) identified the floor type and watering system as the main environmental factors influencing infection rates in cattle. Reshi and Tak (2014) found lower *Eimeria* spp. infection probabilities in cattle herds kept with cemented floor and watered with tap water compared with cattle kept on non-cemented floor and watered at open ponds. Non-cemented floor accumulates urine and increases the temperature providing a warm and wet environment that is favorable for oocyst sporulation (Reshi and Tak, 2014). In Bangalore, the floor of the sheds changed from mostly concrete floors in urban areas to sandy floors in rural areas, which may favor coccidiosis in rural areas. Moreover, Ramachandra and Bharath (2016) found that 80% of the water bodies along the Bangalore boundaries have disappeared from 1973 to 2015, encouraging the use of tap water for livestock in urban areas (Ahirwar et al., 2010). Furthermore, the increased *Eimeria* spp. infection probability and OpG in rural areas might be explained by a higher number of calves sampled in rural areas (15.9%) compared to urban (9.6%) and mixed areas (8.1%), since *Eimeria* spp. infections are more

frequent in calves compared to adults (Cornelissen et al., 1995; see own results section 3.5). However, the interaction effect between SSI and cattle physiological status was not statistically significant in our analyses. Urban dairy farms have developed a more efficient dairy management through a high commercial orientation (Manivannanan and Tripathi, 2007), explaining the increased presence of lactating cows compared to non-productive young cattle in rural areas. As a further justification for the increased *Eimeria* spp. infection probability and OpG in rural areas, we hypothesize that an improved immune-competence exists in cattle kept in urban and mixed areas, since these are more strongly exposed to environmental and infectious disease stressors. Concomitantly, French et al. (2008) investigated variations in stress and innate immunity in tree lizards across an urban-rural gradient. They identified improved response and suppressed stress hormone levels in urban lizards. Pinto et al. (2020) described an increase in ambient shed temperature and rectal temperature of cattle in rural areas due to the scarcity of shadow and heat insulation compared to urban areas. Chronic heat stress causes immune suppression in animals and increases disease susceptibility (Sophia et al., 2016). Major impacts are observed on calf immunity. Nardone et al. (1997) observed a decrease in immunoglobulin A and G concentrations in colostrum from heifers exposed to high temperature during late pregnancy and early postpartum period, which could be an explanation for depressed immune response and resistance to parasite infections in calves and heifers in our rural study areas.

Regarding the impact of season on endoparasite infection, Jithendran and Bhat (1999) identified July-September as the period with the highest risk of GIN infection and pasture contamination in the humid North West Himalayan region of India, indicating that the nematode egg load reached a threshold pathogenic level during the monsoon and autumn seasons. In our study, EpG and OpG were highest in autumn, which may be explained by the humid weather conditions in autumn. Maharana et al. (2016) found an increase of coccidian prevalence in monsoon season followed by autumn and winter, but the data did not statistically differ as is the case in our analysis. We identified the lowest GIN prevalence, EpG and OpG in the summer season, which may be related to increased larval mortality due to high temperature and UV radiation in summer (Dijk et al., 2009). The more favorable climate conditions in monsoon and autumn (i.e. rainfall, humidity) enable the transmission of pre-parasitic larval stages of the parasites from feces to the surrounding herbage and thus increase infection rate (Fiel et al., 2012).

According to our data, the highest percentage of crossbred cattle are kept in urban areas, where pasture access is more frequent compared to mixed and rural areas. Studies conducted under tropical conditions suggested that crossbred cattle are more resistant to GIN infection than purebred *Bos taurus* or *Bos indicus* (Oliveira et al., 2009). Hence, farmers are encouraged to keep crossbreeds in urban areas since they are well-adapted to harsh environments. The lowest (not significant) GIN infection probabilities determined in crossbred cattle were in line with the results of Oliveira et al. (2009). Nevertheless, exotic breeds (i.e. Jersey and Holstein Friesian) are the most common dairy cattle type in Bangalore, as they were imported into India in the 1950s to improve milk yields (Wakchaure et al., 2015). They are less adapted to harsh environmental conditions with a higher susceptibility to helminthiasis, in contrast to Zebu cattle (Hansen, 2004; O'Kelly, 1980; Peña et al., 2000). Although the breed effect was not statistically significant in the model analyses, the *Eimeria* spp. infection probability was highest in the rural area and coincided with the increased percentage of exotic cattle in rural farms.

As expected, infection probabilities and egg/oocyst counts for both GIN and *Eimeria* spp. were significantly higher in calves and heifers compared to lactating and dry cows due to the adaptive immune response in adult cattle (Cornelissen et al., 1995; Matjila and Penzhorn, 2002). Generally, EpG values are known to be low in dairy cows (Borgsteede et al., 2000; May et al., 2017). However, the values in the present study ranged from 0 to 1900 EpG. Only 10 animals had values above 1000 EpG, seven of them were calves or heifers and the remaining three animals were two lactating and one dry cow. Values for OpG are usually higher than for EpG (Pinilla León et al., 2019). Although our values ranged from 0 to 15000 OpG, only 15 animals (14 calves and one heifer) had values above 2000 OpG. In agreement with Waruiru et al. (2000), OpG values were significantly higher in calves and heifers than in adult cows. Assuming *Eimeria bovis* as the most common *Eimeria* species in cattle, Ernst et al. (1984) detected an average of 1151 OpG in calves and 19 OpG in adult cows for *Eimeria bovis*, which agrees with our LSMeans for OpG in calves (1121.5) and adult cows (14.6 for lactating and 20.3 for dry cow).

We identified GIN infections in 41.7% and *Eimeria* spp. infections in 25.6% of all individual cattle. Accordingly, Krishna Murthy and Souza (2016) and Marskole et al. (2016) reported prevalences about 40% for GIN infections and 25% for *Eimeria* spp. infections in Indian dairy cows. Strongylid eggs were the predominant egg morphotype in our study (40.5%), followed by coccidian oocysts (25.6%), *Strongyloides papillosus* (2.3%) and *Trichuris* spp. (0.4%). This is in agreement with the study by Krishna Murthy and Souza (2016), who found prevalences of

39.1% for *Strongylida*, 26.7% for coccidian oocyst and 1.4% for *Capillaria* spp. and with recent studies in European dairy and beef cattle (Borgsteede et al., 2000; Kemper and Henze, 2009; May et al., 2017). Co-infections of GIN and *Eimeria* spp. were present in 10.3% of all samples, which agrees with Chaparro et al. (2016) who found coccidia and strongyle co-infections in up to 15% of 1003 examined dairy cattle in the tropics of Antioquia, Colombia.

In the present study, the presence of co-infections of GIN and *Eimeria* spp. was for the first time determined in dependency of a rural-urban gradient. Interestingly, the highest percentage of co-infections was observed in rural areas. Hence, GIN might be better adapted to urban environments with less impairment on the parasite's life cycle compared to protozoan parasites, while survival conditions are favorable for both parasite species in rural environments. Endoparasite infection dynamics will reflect the impact of urbanization on human-livestock relations in future, because the close cohabitation between humans and livestock that results from rapid urbanization will encourage a better cattle management favoring improved animal health.

5. Conclusions

Eimeria spp. infection probabilities and OpG were significantly higher in cattle kept in rural areas compared to cattle from mixed and urban areas. This demonstrated that the consideration of SSI as a novel environmental descriptor allows capturing dairy husbandry system variations associated with social-ecological challenges that affect protozoal infections. Rural farms are characterized by a poor and extensive herd management practice, reflected in a higher number of calves, which contributes to an increased *Eimeria* spp. prevalence. The prevalence of GIN was higher in cows from urban farms, which might indicate pasture overstocking in these areas. Our findings suggest that the close cohabitation of humans and livestock as a result of rapid urbanization encourages a better animal health management, but simultaneously increases the risk for specific parasitic infections depending on the parasite's life-cycle.

6. References

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CHAPTER 6

General Discussion

1. Defining “urban”

The main purpose of this thesis was to analyze the impact of urbanization on production and functional traits from dairy cattle. It was prepared as part of an interdisciplinary research project on social–ecological changes in the course of urbanization. In order to have a common reference system for a large and collaborative project, an urbanization index SSI was created by Hoffman et al. (2017) and used by the project as a novel descriptor to investigate social-ecological components along a rural-urban gradient in Bangalore, India. The SSI, with the combined measures of building density and distance to the city center of Bangalore, provided a simple approach for characterizing the rural–urban gradient and showed dynamic stages of transformation in Bangalore that were spatially defined – as a discrete variable (as urban, mixed and rural classes) or as a continuous variable from 0 (totally urban) to 1 (totally rural).

There is no generally established way of estimating urbanization – partly because the concept of urban is difficult to define, there is no “universal” consensus of what urban means. For example, at the country level, Sweden considers urban area to be a built-up settlement with at least 200 inhabitants and less than 200m between the houses (Ode and Fry, 2006). In Argentina, any settlement with more than 2000 inhabitants is considered an urban nucleus (Bolay, 2020). In the case of India, urban areas are all places with a municipality, corporation, cantonment board or notified town area committee and which also satisfy all the following criteria: i) a minimum population of 5000 inhabitants; ii) at least 75% of the male main working population engaged in non-agricultural pursuits; and iii) at least 400 inhabitants per square km (Census of India, 2011).

There is also no consensus on how to measure urbanization in the academic world and it generally depends on the subject being studied. To study the impact of urbanization in great tits birds (*Parus major*), Perrier et al. (2018) used the surface covered by vegetation, artificial night lighting and measurements of air and noise pollution to calculate the urbanization level. Thompson et al. (2016) considered ground temperature records and remote sensing to measure snow presence in winter since they hypothesized that less snow cover is a trend in urban areas due to an increase in temperatures in the area, which could be affecting the adaptation process of white clover (*Trifolium repens L.*) in urbanizing gradients in Canada.

There is no consensus even among the members of this project on how best to measure urbanization in the city of Bangalore. From the point of view of an economist, Steinhübel (2019) suggested including other small cities or industrial areas (e.g. the satellite cities of the

primary urban center) when considering the ‘proximity to urban nucleus’ definition when designing the urbanizing index, instead of considering only several transects connected to one big city. Reichenbach (2020), from a dairy production point of view, suggested the consideration of access and proximity to markets (direct sale to consumers or through a dairy cooperative) as criteria of urbanization level of a dairy household. Dairy cooperatives are strong in facilitating technological transformations and commercialization, promoting rural development and ensuring food security (Chagwiza et al., 2016); however, they are weak in offering better prices compared with direct sales (as direct sale eliminates intermediaries). Through the chapters presented in this study, it can be seen that close cohabitation between animals and humans seems have generated a series of changes in management in urban areas, either by cleaning, availability to natural resources (water or feed) or spatial constraints, with respect to rural areas. While it was not within the scope of this research to examine an expanded definition of urbanization, it would be interesting to combine the proximity to markets proposed by Reichenbach (2020) and the inhabitants or build-up density proposed by Hoffmann (2017) when defining the level of urbanization of a dairy farm in the future.

2. Social-ecological challenges on herd management

As a novel descriptor, SSI was used to investigate the impact of social-ecological components on production and functional traits. From chapter 2 to 5 of this thesis, variations in dairy cattle farms along the SSI of Bangalore showed associations between dairy farming systems and socio-ecological conditions related to urbanization. This indicates the relevance of the underlying parameters of herd management and environmental conditions.

Management is an important factor for an efficient dairy herd. The main contributors to herd management that influence productive and reproductive performance of dairy cattle are feeding, breeding, health care and cow welfare. The socio-ecological conditions related to urbanization level of the farm location contribute to the differences in the management of dairy farming in Bangalore.

2.1. Breed composition

It is fairly widely accepted that urbanization leads to intensification of production. Vandecastelen et al. (2018) stated that intensification and profitability levels in agriculture practices increase with proximity to an urban center due to lower transport costs and high output prices which are seen as an incentive to intensify production. Gray and Kevane (2001) believed that competition for land leads to intensified agricultural production and increases household

vulnerability to contingencies, especially the poorest ones. The milk yield (MY) results from the chapters 2 and 3 combined with the higher number of lactating animals found in chapter 5, indicate that urban farms are geared towards intensive production. However, the high number of crossbred cows in urban areas, when compared with the high number of exotic cows in rural areas, could indicate: i) an improvement in management and a better use of resources in the urban farms, which has allowed urban dairy production to remain viable under harsh conditions, or ii) that crossbred cows with compromised productions but strengthened functionalities might be a superior genetic merit under harsh environments.

The exotic breeds in Bangalore are primarily Holstein Freisian (HF) and Jersey. Lactation MY in HF cows under subtropical conditions ranged from 2042 to 6557kg, with an average of 3438kg. The average lactation length ranged from 185 to 514 days with a mean of 366.5 days (Usman et al., 2012). Lactation MY for the Jersey breed was 2229kg under subtropical conditions (Lateef et al., 2008) with a mean lactation length of 321 days (Fernando et al., 2016).

The most common native breed found in Bangalore was the Hallikar breed, one of the best draught breeds of southern India, original from Mysore – southwest of Bangalore. Its lactation MY is around 540kg and ranges from 227 to 1134kg, with average lactation days of 285 and 5.7% of fat content (Nivsarkar et al., 2000).

Since HF and Jersey breeds are famous for their high milk productivity, it would be logical to think that those breeds would be common in production-oriented farms, i.e. urban farms. However, that was not the case in Bangalore, where exotic breeds were concentrated in rural areas. This is probably due to the proximity of the dairy cooperatives that provide artificial insemination (AI) services. The lack of cooperatives in urban areas means that farmers have to rely on private veterinarians (Gizaw et al., 2016), who are more expensive. This would increase expenditure on inputs and negatively affect the final profit. If economic motivation is the most important factor for the management efficiency of urban dairy farmers as suggested by Manivannanan and Tripathi (2007), the high price for AI by private veterinarians might have made them turn to another available source of insemination such as uncontrolled natural mating. In India, bulls and cows are allowed to freely roam the city due to the social and cultural status of the cow which is considered sacred. Thus, the high number of bulls on the streets and the common practice of letting their cattle pasture in the city (chapter 3 and 4) make it more common to have uncontrolled breeding, which would also explain why the number of crossbreeds is higher in the urban area.

In addition to the economic motivation, the good performance and adaptation to the environment could have motivated the growth of crossbreeds in urban areas. The MY production of the crossbreeds varies depending on the ratio of genetic influence of the exotic/native breeds crossed with the environment they live in. The first filial generation (F1) progeny between exotic and native dairy cattle shows improvements in terms of milk production, calving interval and age of sexual maturity. Thus, a F1 between HF and the Indian breed Sahiwal (average MY of 2226 kg) could produce a lactation MY of 2729.9 kg, with a lactation length of 326 days. However, a F1 of HF with Tharparkar (average MY of 1749 kg) could produce 3700 kg of milk per lactation (Mishra et al., 2017; Nivsarkar et al., 2000). To maintain this performance in subsequent generations, it is required a precise breeding program along with a good AI infrastructure. However, the lack of pedigree records in Bangalore and spontaneous mating with unknown bulls make the implementation of a breeding strategy almost impossible at present, compromising the long-term stability of any breeding program.

In tropical areas it is wrong to think that there is a linear relationship between the level of crossbred and performance capacity (Syrstad, 1996). In his review on dairy cattle crossbreeding in the tropics by Rege (1998), he studied the relationship between dairy performance and the proportion of exotic genes. Rege reported that crossbreeds with 50% or above exotic genes did not show big differences for MY, lactation length and age at first calving. Only calving interval was higher with percentages of exotic breeds above 50%, coinciding also with the results of Syrstad (1996). Breeding policy strategies for genetic improvement of dairy cattle in India recommended up 75% of exotic genes for crossbred dairy cattle (Sreenivas, 2013) and the same recommendation was made for another tropical country, Ethiopia (Roschinsky et al., 2015).

In the chapters 3, 4 and 5 we observed a higher percentage of exotic breed cows in rural areas, which as we have already mentioned are more productive than native ones and crossbreeds. However, rural areas, despite presenting better productive genetics, are not producing more MY than urban areas. So this high production of milk from genetically less productive animals in urban areas seems to be a result of other factors, such as management or environment.

2.2. Hygienic and health conditions

The cohabitation of animals and humans in a megacity is a constant challenge. The increasing difficulty in reaching exterior plots increases the number of herds confined indoors, leading to overcrowded stables with high temperatures and humidity. This is a favorable environment for diseases if the farmer does not maintain a high level of hygienic conditions (Daburon et al.,

2017). Vaarst et al. (2007) observed differences in the hygienic management of dairy cattle in (peri-)urban and rural areas of Jinja, Uganda. Cleaner sheds were observed in (peri-)urban (93%) compared with rural (55%) dairy farms. Moreover, clean shed floors made of concrete were mostly found in (peri-)urban farms (60%) while dirt and mud floors were more common in rural areas (71%). Bainesagn (2016) in a survey of 180 dairy farmers from urban, (peri-)urban and rural areas in Ethiopia, found differences in the frequency of cleaning the sheds. All the urban farms were cleaned daily. Most of the (peri-)urban farms preferred daily cleaning too but 19% cleaned weekly. In the rural area daily cleaning was the common management practice (93%), however 5% did not clean at all. Accordingly, chapter 3 showed superior hygiene scores for dairy farms located in urban areas, indicating increased attention to hygienic and health conditions.

The context of inadequate sanitary conditions and the recent evolution of human activities (e.g. livestock management and use of unsafe water) also gives rise to problems of disease incidences and zoonosis (Sabourin et al., 2018), which are intensified in areas with dense human-animal cohabitation such as urban areas. Some of the most important and well-known human zoonosis are caused by gastrointestinal parasites such as the zoonotic agent *Fasciola* spp. that is widely prevalent in Indian cattle (Gupta and Singh, 2002). The study conducted by Mhoma et al. (2011), about gastrointestinal nematode (GIN) prevalence in goats in Mwanza City, Tanzania, revealed a higher prevalence of GIN in urban (68.9%) compared with (peri-)urban areas (47.3%). In chapter 5, although it was also shown to have a higher GIN in urban areas (42.5%) compared with (peri-)urban areas (32.9%), the overall prevalence of GIN in Bangalore was ca. 20% lower than in Mwanza city. The limited sanitary conditions of Mwanza City, e.g. limited fresh water lines (75.0% coverage) and even more limited sewage coverage (23.7%) (MWAUWASA, 2013), could explain the elevated prevalence there. However, Bangalore's prevalence is closer to the values found on conventional European farms (41.0%; May et al., 2017) with adequate sanitary conditions, further supporting the hypothesis of better management through social-ecological characteristics in urbanizing environments.

2.3. Feed efficiency

Controlling parasite infections can have direct effects on the farm economy through improvements in health and cattle performance (i.e. productivity, fertility and weight gain) (Charlier et al., 2009) but can also have indirect effects on CH₄ emissions through feed efficiency (Fox et al., 2018). Reichenbach (2020) found out that higher efficiency in the conversion of ingested feed into milk was indicative of farms that relied on higher quality feedstuff, public

ground for pasture and organic wastes from markets which is in line with the management of our urban farmers in Bangalore. Thus, different feeding management through the rural-urban gradient of Bangalore affected GHG emissions showing an inverse relationship between GHG emission and urbanization level.

2.4. Environment related to enteric methane emissions

In chapter 4, we observed that variations in dairy husbandry feeding, as well as management strategies, can affect enteric CH₄ along the rural-urban gradient of Bangalore. However, as we also observed in that chapter, in Bangalore there are several contributing factors in addition to the level of urbanization which make up the SSI; these include feeding strategy, environmental conditions and measurement location. The variability of these factors makes it difficult to measure, analyze and find solutions to mitigate CH₄ emissions. To overcome this limitation in the future, it would be necessary to improve and standardize the on-farm CH₄ measurements. For example, by doing measurements outdoors avoiding solar radiation and wind interaction to minimize reflectance and dissipation of CH₄ (Chagunda et al., 2013) and limiting the interval between feeding and CH₄ recording, for example to between 3 to 5 hours as suggested by Reintke et al. (2020).

3. Genotype by environment (G×E) interaction

The breeds studied in this research were either endemic to Bangalore (the Hallikar, that is adapted to the tropical savannah climate) or from foreign temperate-zone regions (i.e. HF or Jersey). G×E interaction is extremely important in cattle breeding in the tropics. The increasing demand for dairy products in developing countries makes it necessary to have a better understanding of G×E interaction to identify appropriate genotypes to optimize dairy production systems (Roessler et al., 2019). Local breeds from the tropics and subtropics are well adapted to harsh climate conditions but have a very low milk production, while on the other hand, high-producing dairy breeds from the temperate zones, despite their high genetic potential for high MY, are not well adapted to the harsh tropical conditions, and thus do not perform satisfactorily, resulting in reduced MY due to heat stress (Javed et al., 2004).

The existence of possible G×E interaction in different urbanization levels for production or functional traits has not been studied yet, so if traits act differently in different environments, a re-ranking of genotypes can be expected, which could lead to a rethinking of current breeding objectives.

3.1. Production traits

In this research, MY and body condition score (BCS) were the two production traits analyzed and interacted with different environments (urbanization level). Shabalina et al. (2020) also studied production traits (MY and fat percentage) exposed to different production systems (conventional and organic) however no G×E interaction was founded. In Figure 6.1(a) for MY, all genotypes (i.e. Exotic, Crossbreed and Native) decreased along the SSI, however more decreases were found for Native breeds. This is because those breeds in rural areas are used for both milking and dragging purposes, while they are only used for dairy purposes in urban areas.

The phenotype BCS represented in Figure 6.1(b) shows also decreases in BCS across SSI. Opposite to MY, Native always had the best BCS and a small G×E interaction was observed for the genotypes Exotic and Crossbreed, having similar estimates in urban areas but differing in (peri-)urban and rural areas. Those results suggest only a small G×E interaction for production traits between urban and other environments.

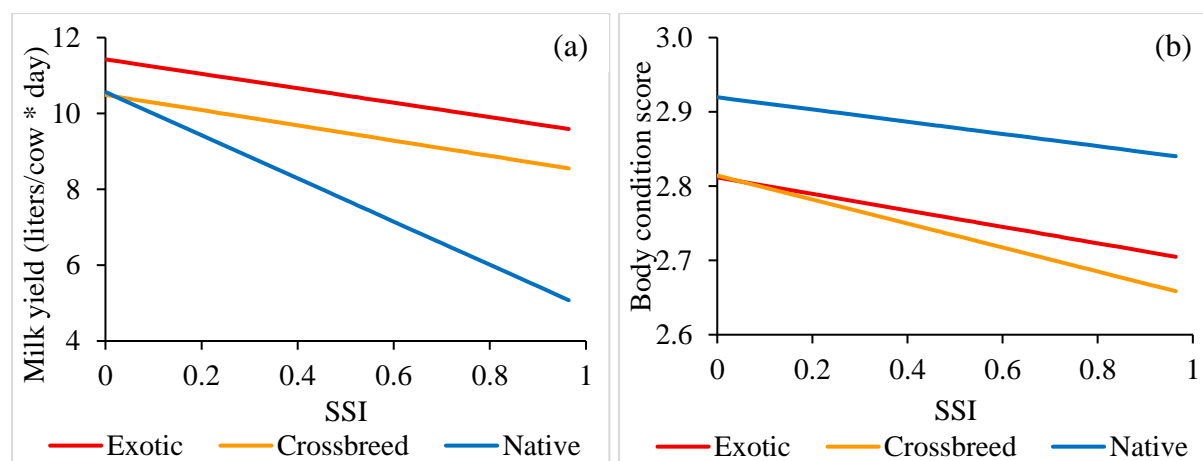


Figure 6.1. Genotype × environment interaction. (a) Milk yield (liters/cow * day) and (b) body condition score for three genotypes (Exotic, Crossbreed and Native) along the Survey Stratification Index (SSI).

3.2. Health traits

Leg health traits, including hock assessment score (HAS) and locomotion score (LS), were studied in chapter 3. Fatehi et al. (2003) studied leg health through hoof and leg conformation traits and concluded that a certain level of G×E interaction existed for hoof and leg traits evaluated in two housing systems (free and tie stall). Similarly, in Figure 6.3(a), a clear G×E interaction was manifested between the genotypes Exotic and Native for the phenotype HAS. While in Figure 6.3(b) for the phenotype LS, G×E interaction were founded for Crossbreed with the rest of the genotypes, highlighting a significant interaction (P -value = 0.008) between

Exotic and Crossbreed. Those results suggest a clear G×E interaction existence for leg health traits.

Other cattle health traits studied were subclinical mastitis (SubMast) and rectal temperature (RT). Shabalina et al. (2020) reported a G×E interaction for the incidence of mastitis between cattle kept in conventional and organic production systems. Similarly, in Figure 6.3(c) and (d), a clear G×E interaction were observed for SubMast and RT between the three genotypes. Surprisingly, the Native genotype showed the highest SubMast incidence and RT in rural areas and the lowest SubMast incidence and RT in urban areas. However, the low number of animals sampled with Native genotype could be affecting this result. Nevertheless, the Crossbreed genotype performed better in rural environments in terms of health traits, except for HAS.

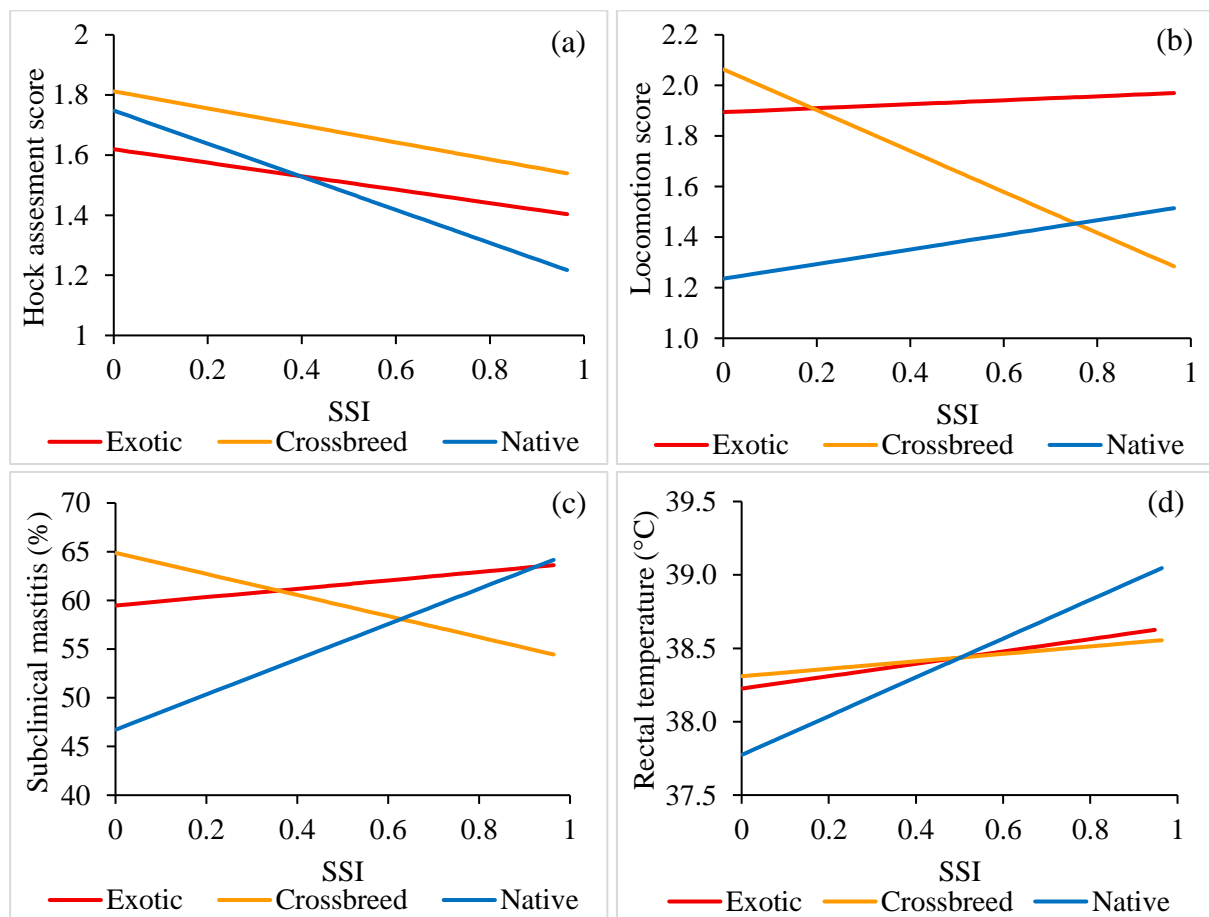


Figure 6.2. Genotype × environment interaction. (a) Hock assessment score, (b) locomotion score, (c) subclinical mastitis probability (%) and (d) rectal temperature (°C) for three genotypes (Exotic, Crossbreed and Native) along the Survey Stratification Index (SSI).

3.3. Methane emission

In his study, Chagunda et al. (2009) found a significant G×E interaction on enteric CH₄ production considering two management systems (high and low forage access) and two genotypes (high and low fat and protein milk composition). Similarly, in our study, different

genotypes were tested for different environments along the SSI of Bangalore. Figure 6.4 showed a re-ranking of genotypes for AllMean phenotype also indicating a G×E interaction for CH₄ emission across the SSI environment. The Exotic genotype did not show obvious environmental variations while Crossbreed and Native genotypes increased their emission in urban environments. Those results suggest that Exotic genotypes are better to cope with CH₄ emission in an urbanizing environment.

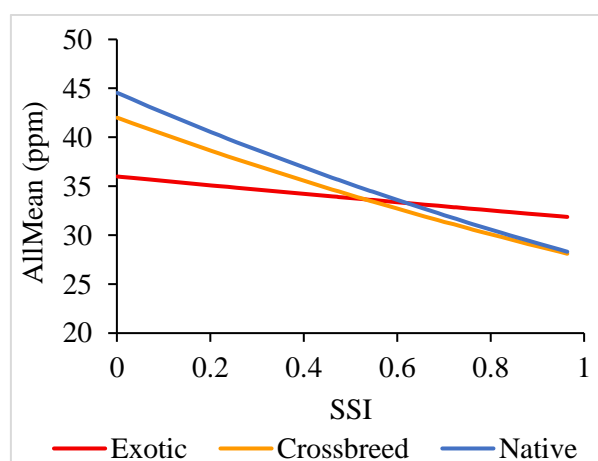


Figure 6.4. Genotype × environment interaction. Means in absolute values of overall CH₄ (AllMean, including respiration and eructation measures) for three genotypes (Exotic, Crossbreed and Native) along the Survey Stratification Index (SSI).

3.4. Endoparasite

Baker et al. (2004) demonstrated a G×E interaction between two sheep genotypes (resistant and susceptible to nematode infection) in environments with different challenge levels (high and low). In that study, breed differences were more obvious in the high nematode challenge environment (favoring the resistant genotype) and almost disappeared in the low challenge level environment. A G×E interaction for endoparasite infection has been also found for dairy cattle in Figure 6.5, which shows a re-ranking of genotypes for eggs and oocyst count per gram of feces across the SSI environment. The Native genotype seems to have a higher sensitivity to environmental changes than the other two genotypes for health traits and therefore for the endoparasite infection as well. Similar to other health traits previously mentioned, Crossbreed genotypes performed better in rural environments than Exotic genotypes.

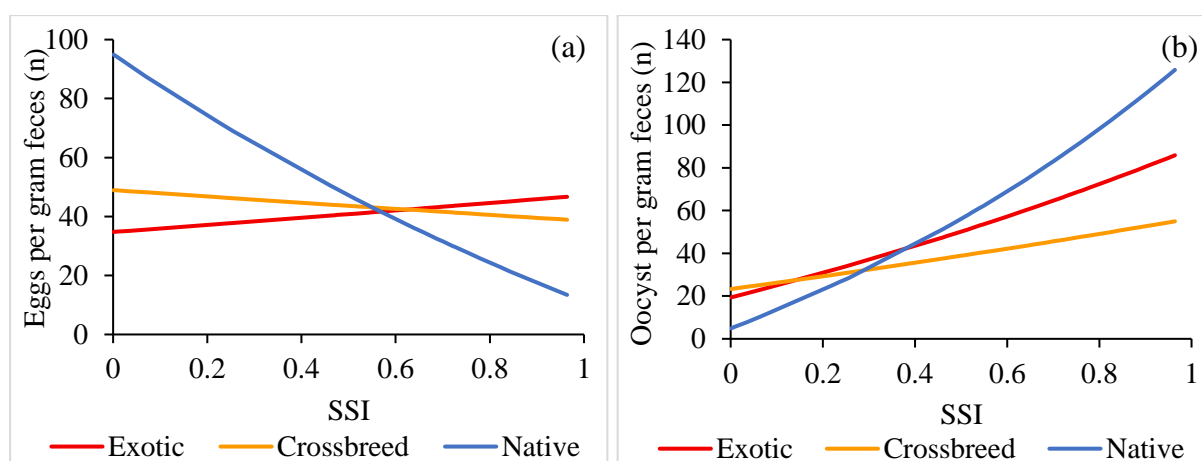


Figure 6.5. Genotype × environment interaction. (a) Eggs per gram feces (n) and (b) oocyst per gram feces (n) for three genotypes (Exotic, Crossbreed and Native) along the Survey Stratification Index (SSI).

4. Environmental condition descriptors on productivity and functionality

In this study, SSI was considered a novel environmental descriptor to define the level of urbanization in the rural-urban gradient of Bangalore. In addition, this thesis studied other factors that influence the productivity and functionality of dairy cattle, e.g. housing conditions and temperature-humidity index (THI).

4.1. Survey stratification index (SSI)

Through the chapters 2 to 5, the SSI of Bangalore influenced production and functional traits of dairy cows, which revealed the importance of the internal socio-ecological structure of the rural–urban interface in Bangalore. In chapter 2 and 3, better production traits (MY and BCS) and hygienic conditions although poorer leg health in urban compared to rural areas reflected a production-oriented dairy system through better management but at the detriment of animal welfare.

Past studies in Uganda and Ethiopia (Bainesagn, 2016; Vaarst et al., 2007) have found that urban dairy farms draw their water from taps (100%) while rural dairy farms primarily used local water sources – drawing from local springs, ponds or rivers (83-91%). This may be due to differences in infrastructure or access issues in the different environments. Thus, the higher access to tap water in urban areas in India (Balasubramaniam et al., 2014) could explain the better management and hygiene found there. Moreover, water origin is one of the main environmental factors influencing both milk contamination (Zhou et al., 2019) and endoparasite infection (Rehman et al., 2011), which may have influenced our findings in chapter 5 of rural farms exhibiting an increased prevalence of *Eimeria* spp.

4.2. Housing conditions

In a study focused on 120 dairy farmers in the rural Bangalore, 80% of the farmers counted with stone slabs as flooring material on the cattle location, although 33% of the farmers did not have a shed to house their cattle, so they tied them up under a tree or next to the house (Chandrasekar et al., 2017), which seems to be related to the higher heat stress due to shadow scarcity as shown in chapter 3.

The other housing condition that can impact animal productivity and functionality, specifically locomotion health (Norrington et al., 2008; Singh et al., 1993) and endoparasites infection (Rehman et al., 2011; Reshi and Tak, 2014), is the flooring material. The ground floor shed, typically found in indoor livestock farming can lead to overcrowded stables with high temperature and humidity affecting animal health, welfare and MY performance (Daburon et al., 2017).

4.3. Temperature-humidity index (THI)

THI has been used to indicate animal heat stress, characterized by decreased productivity and impaired functionality. Bohlouli et al. (2019) reported a G×E interaction for milk performances at moderate and extreme THI when considering heat stress conditions. McDowell (1958) reported an increase in RT related to a rise in ambient temperature. Similarly, in chapter 3 a significant impact of ambient temperature on RT (*P-value* < 0.001) was observed. In the same chapter, ambient temperature and humidity also showed a significant impact on udder hygienic score (UddHS) and upper leg hygienic score (ULHS) respectively (*P-value* = 0.01 and 0.04 respectively). Methane emissions, in particular the eructation CH₄, were significantly influenced by THI (chapter 4). Furthermore, in chapter 5, an interaction between SSI and THI was noticed for the binary endoparasite infection (BinEpG/BinOpG) although temperature was the only factor showing an interaction when temperature and humidity were considered separately (Table 6.1). Consequently, in genetic evaluations of production and functional traits, heat stress and its interaction should be considered when the urbanizing environment is described.

Table 6.1. *P-values* for the interaction between the Survey Stratification Index (SSI), temperature (Temp), humidity (Humid) and Temperature and Humidity Index (THI) for the endoparasites presented in the chapter 5.

Endoparasite	SSI*THI	SSI*Temp	SSI*Humid
BinEpG	0.02	0.01	0.64
BinOpG	0.1	0.02	0.46
EpG	0.55	0.07	0.09
OpG	0.33	0.36	0.99

BinEpG = Infection probability of gastrointestinal nematodes; BinOpG = Infection probability of *Eimeria* spp.; EpG = Eggs per gram feces for gastrointestinal nematodes; OpG = Oocysts per gram feces for *Eimeria* spp.

5. Future perspectives and further research

This study focuses mainly on the effects of the rapid growth of megacities like Bangalore on productivity and functionality of dairy cattle. These cities are expected to dominate future urbanization trends and present new challenges for natural resources and agricultural systems. The unique anthropogenic disturbance created by the cities, considered as new ecosystems, has led to changes in evolutionary processes of microbes, plants, animals that inhabit cities (Johnson and Munshi-South, 2017). Caizergues et al. (2018) revealed a strong phenotypic differentiation when comparing morphological features between urban and forest great tits birds (*Parus major*). Winchell et al. (2016) found phenotypic shifts in urban areas in the tropical lizards of Puerto Rico. Urban lizards showed longer limbs and more subdigital scales or lamellae, compared to lizards from forested habitats as a result of adaptation to an urbanized environment. In developing countries, where urbanization has spread considerably quickly, evolutionary or adaptive processes have been observed in livestock production systems as well (Daburon et al., 2017). Increasing urbanization could open opportunities for considering novel environmental effects in dairy farming to create sustainable production and meet social demand (Boichard and Brochard, 2012).

The variations in dairy farming systems have been shown to be associated with the socio-ecological conditions related to urbanization. Thus, the use of an urbanization index such as SSI would allow creating more complete and accurate socio-ecological models for the analysis of livestock production systems. Knowing the socio-ecological conditions and the G×E interaction of an urbanizing environment could improve genetic selection for better adaptation to urban areas.

The present thesis has been based on spatial data when considering different locations in a rural-urban gradient. However, urbanization and livestock systems change over time in response to changes in rural human population density, production demand and the agro-climatic environment. The simultaneity of these processes makes it difficult to establish cause-effect relationships in spatial data analysis without making strict or unrealistic assumptions (Steinhübel, 2019). To overcome this limitation, it would be advisable to follow up with the dairy farms already studied in this project and check how they evolve and change in time and space as a way of understanding and improving the sustainability of an urban landscape, as advisable by Wu (2014). For example, as urbanization progresses, it would be interesting to know whether i) the (peri-)urban dairy farms apply similar management to those already observed in urban areas and tend towards production-oriented systems, ii) they opt for other new strategies, or iii) they stop their dairy activity; this would improve our understanding of the limitations and opportunities offered by urbanization and the factor combinations that allow dairy production to remain viable at different locations along this rural-urban interface. It would also be interesting, as suggested by Reichenbach (2020), to include in the study more remote rural dairy farms where there is no nearby urban nucleus or dairy cooperative since low market integration seems to favor non-production oriented systems and native livestock breeding compared to other settlements (Reichenbach, 2020).

For urban and (peri-)urban agriculture to continue, and thus further contribute to the local economy, to food security and to the food supply in an urbanizing environment, the welfare conditions of dairy cattle need to be improved with adequate open sheds with soft flooring materials. The authorities could also help by providing a solution for the ownerless bulls, AI access, fodder scarcity and the theft of dairy cattle that damages the economy of the farmers (in Puttanahalli, an urban settlement, farmers complain of continuous theft of their cattle). Moreover, to maintain proper sanitary conditions for farmers, animals and neighbors, a manure removal process and adequate sewage needs to be provided in urban areas.

6. Conclusions

This study showed that the impact of urbanization on production and functional dairy cattle traits are complex and related to social-ecological components. Moreover, urbanization in Bangalore tends to improve the management and production of dairy cattle but at the detriment of certain health traits like locomotion.

The variations along the rural-urban gradient of Bangalore on dairy cattle traits showed the relevance of parameters like herd management and environmental conditions. Thus, the use of an urbanization index such as SSI would allow creating more complete and accurate socio-ecological models for the analysis of livestock production systems in urbanizing environments.

The present study also demonstrated the need to know the characteristics of the environment through socio-ecological descriptors in order to find the genotypes that are best adapted to each level of urbanization, since we observed the existence of a genotype by SSI environment interaction for functional traits in dairy cattle in Bangalore. These results highlight the need to consider G×E interaction in dairy cattle breeding in urban environments and thus identify appropriate genotypes to optimize dairy production systems to build sustainable, efficient and resilient livestock systems in urbanizing environments.

7. References

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„Ich erkläre Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlichgemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

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Gießen, den April 2021

Ana Pinto García